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CERTIFICATE

THIS IS TO CERTIFY THAT THE DISSERTATION ENTITLED **"PROFILE OF VENOUS THROMBOEMBOLISM IN INDIA"** IS A BONAFIDE WORK OF **DR. RAJASEKAR.T**, IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE D.M. BRANCH X (CLINICAL HAEMATOLOGY) EXAMINATION OF THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY, TO BE HELD IN AUGUST 2008.

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CONTENTS

ABSTRACT

Until the 1990s, venous thromboembolism (VTE) was viewed primarily as a complication of hospitalization for major surgery or major medical illness. However epidemiologic studies have shown that between one quarter and one half of all clinically recognized symptomatic VTEs occur in individuals who are neither hospitalized nor recovering from a major illness. Evaluations of risk factors in this group of patients help to identify high-risk patients who could benefit from prophylaxis. In this study, we evaluated such risk factors in patients referred for thrombophilia risk screening. 266 patients with documented deep vein thrombosis were evaluated for thrombophilia risk factors. Median age of presentation is 38 years (4-74) and only 4/266 (1.5%) were below 15 years of age. History of recurrence and family history was present in 38/266 (14.3%) and 10/266 (3.8%) of patients, respectively. Male: Female ratio was 1:1.1 (140/126) for the whole group. Males were more commonly affected than females in the subgroups also, except for patients with cortical sinus thrombosis, where male: female ratio was 0.6:1. The presenting features were lower limb deep vein thrombosis in 123/266 (46.2%) patients, followed by cortical sinus thrombosis in 92/266 (34.6%), pulmonary embolism in 26/266 (9.8%) and Budd Chiari syndrome was seen in 20/266 (7.5%) of patients. 121/266 (45.5%) of patients were on anticoagulant therapy at the time of evaluation. Prothrombotic risk factors were present in 163/266 (61.3%) of patients and no risk factors were identified in 103/266 (38.7%) of patients. More than one risk factor was seen in 104/266 (39.1%) of patients. The most common risk factor identified in these patients was an elevated factor VIII level- seen in 159/266 (59.7%) of patients. Factor V Leiden mutation was seen in 16/203 (7.9%) patients, Prothrombin G20210A mutation in 2/203 (1.0%) and MTHFR Cys677Thr homozygous mutations in 7/203 (3.4%) of patients evaluated for these parameters. In patients with Budd Chiari syndrome the prevalence of Factor V Leiden mutation was 13.3%. Protein C, S and antithrombin deficiency was present in 7/266 (2.6%), 13/266 (4.8%) and 20/266 (7.5%) respectively.

INTRODUCTION

Venous thrombosis, comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), occurs with an incidence of approximately 1 per 1,000 annually in adult populations.[1] The major outcomes of venous thrombosis are death, recurrence, post-thrombotic syndrome, and major bleeding due to anticoagulation. Thrombosis is also associated with impaired quality of life, particularly when post-thrombotic syndrome develops.[2,3] Death occurs within 1 month of an episode in about 6% of those affected with DVT and 10% of those with PE.[4] The mortality rate for PE has been estimated to be high as 30% in studies that included autopsy-based PE diagnosis,[5] emphasizing that many PE are not recognized clinically before death. Although knowledge of the pathogenesis of venous thromboembolism has advanced markedly over recent years, how best we use this knowledge to improve outcomes in clinical practice raises important questions. It has become common practice for individuals presenting with suspected venous thromboembolism to be subjected to a variable range of laboratory tests for the identification of heritable and acquired prothrombotic states, or thrombophilia. While superficially this may seem to be an entirely reasonable approach, evidence that the results of some such investigations should guide clinical management is incomplete.

Cerebral vein thrombosis (CVT) is a rather uncommon thrombotic disease. However, the recent introduction of noninvasive and highly sensitive diagnostic techniques such as magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), and computed tomography angiography (CTA) has modified our knowledge of the spectrum of illness associated with CVT.

This study aims to study the profile of patients diagnosed with deep vein thrombosis in CMC, Vellore.

REVIEW OF LITERATURE

Introduction

The clinical definition of thrombosis is that of the pathological presence of a clot (thrombus) in a blood vessel or in the heart that causes the obstruction of blood flow through the circulatory system. Depending on the location where thrombus formation takes place (i.e. in the venous or arterial part of the vessel tree), thrombotic disease can be classified into venous and arterial thrombosis. Both types of thrombosis are considered as distinct disease states that are characterized by different pathogenic mechanisms and underlying risk factors.

Venous thromboembolism (VTE), comprising deep-vein thrombosis (DVT) and pulmonary embolism (PE), is associated with substantial morbidity and mortality. Detailed estimates of the annual number of VTE events are hard to obtain because VTE is difficult to diagnose. This is due to a number of factors; VTE is often clinically silent and, in many cases, the first sign of the disease is a sudden fatal PE.[5,6]

The chronic nature of VTE and its recurrences and complications requires considerable healthcare resources for its management. Additionally, morbidity and healthcare costs are incurred from associated complications of VTE, such as post-thrombotic syndrome (PTS), which affects at least one-third of DVT patients[7,8,9,10,11], and pulmonary hypertension (PH), which occurs in 4%–5% of patients following PE[12].

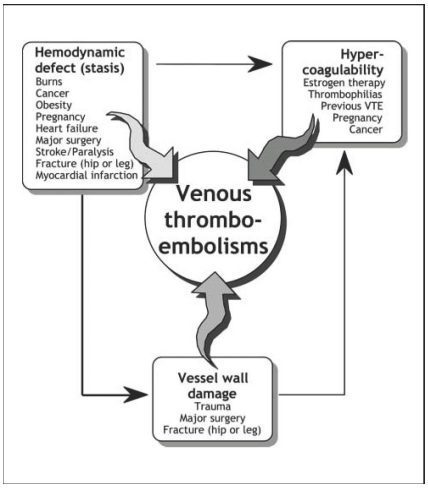
Pathophysiology

Central to the pathogenesis of both venous and arterial thrombosis is the perturbation of the normal haemostatic balance. In healthy individuals, haemostasis is carefully controlled by several anticoagulant mechanisms that prevent inappropriate vascular blood clotting. Several observations are even in favor of a slight dominance of the anticoagulant forces during homeostasis.[13] This dynamic equilibrium between pro- and anticoagulant factors can be rapidly shifted in favor of coagulation, in case of a physiological need for cessation of blood loss. To prevent excessive blood loss, the hemostatic system, which includes platelets, endothelial cells, and plasma coagulation proteins, is called into play. Immediately after tissue injury, a platelet plug is formed through the processes of platelet adhesion and aggregation. Blood coagulation may be considered a mechanism for rapid stabilization of an otherwise unstable platelet plug with a fibrin clot. A series of interdependent enzyme-mediated reactions translates the molecular signals that initiate blood coagulation into a major biologic event - the formation of the fibrin clot. However, this proneness towards clot formation implies a risk to develop thrombosis.

In 1884, Rudolph Virchow, the German pioneer in the field of haemostasis, postulated that three major causes contribute to thrombus formation (Virchow's triad) (fig: 1)

- Vascular endothelial damage
- Stasis of blood flow
- Hypercoagulability of blood.

Figure-1



Vascular endothelium

The normal, unperturbed endothelium represents a dynamic interface between the flowing blood and the vessel wall. The endothelium regulates the fluid state of blood, vascular tone, inflammatory processes, vascular permeability, and vascular fragility. In its basal state, the endothelium presents a non-thrombogenic surface to the circulation by inhibiting platelet aggregation, preventing the activation and propagation of coagulation, and enhancing fibrinolysis[14]. These activities are accomplished by both passive and active processes.

Inhibition of platelets

When in close proximity to endothelial cells, platelets become unresponsive to agonists. This inhibition of platelet aggregation is accomplished by the secretion of prostaglandin I_2 (PGI_2), or prostacyclin, and nitric oxide (NO), and by the surface expression of an ecto-ADPase identified as CD39.[15] Prostacyclin is synthesized mainly by vascular endothelial and smooth muscle cells as a product of arachidonic acid metabolism. It inhibits platelet activation, secretion, and aggregation and monocyte interactions with endothelial cells. It

also causes vascular smooth muscle cell relaxation. NO similarly has a wide range of functions, including the inhibition of platelet adhesion, activation, and aggregation. Most of the NO released from endothelial cells is elaborated abluminally, where it acts on the smooth muscle cell to cause vasodilation. Some NO also may enter the lumen, however, and from there diffuse into platelets. Prostacyclin and NO can act synergistically to reverse platelet aggregation[16]. The released platelet agonist adenosine diphosphate (ADP) can be inactivated by endothelial membrane-associated CD39 by metabolism of ATP and ADP to cyclic adenosine monophosphate (cAMP). This eliminates platelet recruitment and returns the platelets to their resting state. ATP and ADP can stimulate purinoreceptors on endothelial cells, resulting in synthesis and release of PGI_2 and NO.[17]

Inhibition of coagulation

Four mechanisms may be utilized by endothelial cells to inhibit coagulation.[16]

(1) Thrombomodulin on the surface of endothelial cells binds thrombin. This coupling inhibits the coagulant properties of thrombin and also increases its affinity for protein C, which it cleaves and activates. The activation of protein C by the thrombin-thrombomodulin complex is augmented by its binding to the endothelial cell protein C receptor.

(2) Protein S, which is thought to be synthesized primarily by the endothelial cell, acts as a cofactor for protein C but also has anticoagulant properties in its own right. Independent of the presence of activated protein C, free protein S is able to inhibit the prothrombinase and intrinsic tenase complexes and interact directly with factors Va and VIIIa.[18]

(3) Heparan sulfate proteoglycans are secreted onto the luminal surface of endothelial cells and into the subendothelium and are capable of binding and activating antithrombin, thereby accelerating the inactivation of several procoagulant serine proteases including thrombin, factor Xa, and factor IXa.

(4) Tissue factor pathway inhibitor (TFPI) is synthesized in the liver and in endothelial cells and has been shown to be localized to apical granules of the endothelial cell. The tissue factor-factor VIIa-factor Xa complex is inhibited by TFPI.

Blood Stasis

Venous stasis represents an important pathogenic factor in the development of PE. The role of venous stasis has been investigated in patients with spinal cord injury and other forms of paralysis. These studies show that the majority of venous thrombi originate in regions of slow blood flow, e.g., the large venous sinuses of the calf and thigh or in valve cusp pockets or bifurcations of the venous system. It has been suggested that blood pooling leads to activation of the coagulation system, thus resulting in a state of local hypercoagulability. In addition, possible endothelial damage from distension of the vessel walls by the pooling blood leads to further activation of the homeostasis system. The activation products of clotting and fibrinolysis can also induce endothelial damage which, in turn, promotes a local state of hypercoagulability.

Hypercoagulability

The risk of venous thrombosis is increased when the homeostatic balance between pro- and anticoagulant forces is shifted in favor of coagulation. When this imbalance is due to

an inherited defect, the resulting hypercoagulable state remains a life-long risk factor for thrombosis. In contrast, hypercoagulability due to a transient factor should be treated only as long as the risk factor is present. In most cases, disturbances in the coagulation cascade arise in inherited thrombophilias.[19] Since each genetic defect represents an independent risk factor for thrombosis, individuals with multiple defects have a significantly increased risk of thrombosis.

Risk Factors for Venous Thromboembolism

In the last century, recognition that all DVT risk factors reflect these underlying pathophysiologic processes and that VTE does not usually develop in their absence has increased. An understanding of the risk factors for venous thrombosis is necessary in order to maximize the prevention of this disease in high-risk individuals and groups of patients. This is because DVT rarely occurs in the absence of risk factors. These risk factors are cumulative in their effect. Usually, more than one risk factor is present in patients with DVT. In one study of patients thought to have DVT, the incidence of abnormal noninvasive test results was 11% when no major risk factors were present, 24% when 1 risk factor was present, 36% when 2 major risk factors were present, 50% when 3 major risk factors were present and 100% when 4 or more risk factors were present[20]. Risk factors convincingly demonstrated for VTE include increasing age, prolonged immobility, malignancy, major surgery, multiple trauma, prior VTE, and chronic heart failure (Table 1)[21]. However, it is important to recognize that the predictive values of these factors are not equal.

Table 1: Risk Factors for VTE[22]

Strong risk factors (odds ratio>10)	Moderate risk factors (odds ratio 2–9)	Weak risk factors (odds ratio <2)
Fracture (hip or leg)	Malignancy	Bed rest >3 days
Hip/ knee replacement	Central venous lines/ chemotherapy	Immobility due to sitting
Major general surgery	Previous VTE	Increasing age
Major trauma	Congestive heart/ respiratory failure	Laparoscopic surgery
Spinal cord injury	Hormone replacement therapy	Obesity
	Oral contraceptive therapy	Pregnancy/ antepartum
	Inherited Thrombophilia	Varicose veins

On the basis of identifiable risk factors and potential causes, VTE may be divided into secondary, primary, and idiopathic VTE. The major risk factors for thrombosis include endogenous patient characteristics such as obesity and genetic factors, and triggering factors such as surgery, immobility, or pregnancy. Some of the risk factors are modifiable, while others, like advancing age and genetic predispositions, are not. Venous thrombosis tends to occur due to the additive effects of endogenous, genetic, and environmental risk factors present simultaneously. This includes the additive effects of environmental risk factors, as illustrated in Fig 2. [22,23]

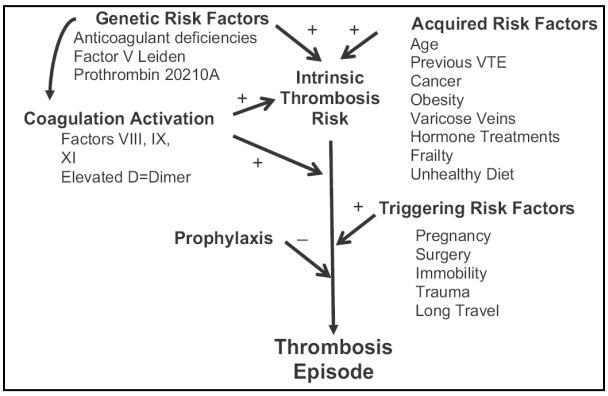


Figure 2 conceptual framework for the interaction of risk factors in development of venous thrombosis. Intrinsic thrombosis risk is defined by the combinations of genetic and acquired risk factors, including modulation of coagulation activation by known and unknown determinants. The intrinsic risk is altered by the occurrence of triggering risk factors, which can be balanced in appropriate settings by use of

Thrombophilia

Approximately 80% to 90% of all causes of thrombosis can now be defined with respect to cause. Of these, more than 50% of all patients harbor a congenital or acquired blood coagulation protein or platelet defect that caused the thrombotic event. It is obviously of major importance to define those individuals harboring such a defect, as this allows:

(1) Appropriate antithrombotic therapy to decrease risks of recurrence

(2) Determination of the time the patient should remain on therapy for secondary prevention, and

(3) Testing of family members in those harboring a blood coagulation protein or platelet defect that is hereditary.

Thrombophilia is defined as a disorder associated with an increased tendency to venous thromboembolism (VTE).[24] The term thrombophilia was introduced as aMeSH (Medical Subject Headings) term in 1998. Thrombophilia can be acquired or inherited.

Acquired thrombophilia

Major General Surgery

The risk of VTE after major general surgery has been extensively documented. The term "major general surgery" is applied to patients who undergo abdominal or thoracic operations that require general anesthesia lasting > 30 minutes.[25,26] Other types of surgery associated with a high risk of VTE include coronary artery bypass,[27,28] surgery for gynecological malignancies,[29,30] and major urological surgery.[24] The risk after neurosurgery is similar.[31]

Major Orthopedic Surgery

Lower extremity orthopedic operations carry a particularly high risk. Without prophylaxis, approximately half of the patients undergoing elective total hip or knee replacement develop VTE. However, only about 5% of these patients manifest symptoms of VTE.[24,32] whereas calf vein thrombi tend to be evenly distributed between the 2 legs in patients recovering from hip replacement, 90% of proximal thrombi occur on the operated side.[33,34] Patients undergoing arthroscopic knee surgery are at low to moderate risk, so VTE prophylaxis is optional.[35]

Spinal Cord Injury

The overall incidence of DVT within 3 months of paralytic spinal cord injury is 38%; the corresponding frequency of PE is < 5%.[36] The risk appears greatest during the first 2 weeks after injury, and fatal PE is rare > 3 months after injury. [37,38] The cause of the decrease in clinically evident PE after 3 months is unknown. However, a number of changes associated with chronic paralysis may be involved, including gradual atrophy of the leg muscles and, in many individuals, the development of small caliber collateral veins around organized old venous thrombi that completely obstruct the deep leg veins.

Fracture of the Pelvis, Hip, or Long Bones

Patients with traumatic hip fracture are at high risk for VTE. The problem was further emphasized in 1959 when the first controlled trial of anticoagulant prophylaxis after hip fracture showed a reduction from 10% to 0% of death because of PE.[39] Patients with fractures of the pelvis or femur are also at high risk. The increased risk after cast

immobilization of tibial fractures is particularly well documented, with an overall VTE rate of 45%, but only one third of those being symptomatic.[40]

Malignancy and VTE

An association between malignancy and VTE was first recognized in 1864 by Armand Trousseau in patients with occult carcinoma and this increased risk of thrombosis due to a combination of factors.[41] From a pathogenic view, patients with cancer often have a hypercoagulable state due to the production of substances with procoagulant activity, particularly tissue factor and cancer procoagulant and tumors can compress veins causing stasis, and cancer patients are exposed to hospitalization, surgery, and chemotherapy, which all increase their risk. Clinical thromboembolism was estimated to occur in up to 10% of cancer patients, and is the second leading cause of death.[42,43] Conversely, approximately 20% of patients with symptomatic DVT suffer from previously established malignancy[44]. However, thromboembolism can precede the diagnosis of malignancy by months or even years[45]. Advanced cancers are associated with a high incidence of VTE, especially cancers of the breast, lung, brain, pelvis, rectum, pancreas, and gastrointestinal tract. Administration of chemotherapy increases risk, like those documented in patients with myeloma on treatment with Thalidomide.[46,47] In addition, women with breast cancer who undergo chemotherapy in association with surgery have 3 times the risk of VTE compared with women who undergo surgery alone[48]. In another study cancer was reported in 39% patients with ultrasound confirmed DVT[49]. Of these, 62% had active cancer, and 38% had a past history of cancer. Of those with active cancer, 54.6% were receiving chemotherapy and 17.3% were receiving radiation therapy at the time of diagnosis of acute DVT. Active cancer patients were younger, likely to be male, and had

a lower average body mass index (BMI). Among the patients with active cancer and documented cancer type, lung, colorectal, breast, lymphoma, brain, and prostate cancer were the most common. Common DVT risk factors among patients with active cancer included surgery within the prior three months, previous cigarette smoking, immobility, hospitalization within 30 days, and prior VTE. However in multivariable regression analysis, only presence of an indwelling central venous catheter and lack of prophylaxis remained independent risk factors for developing DVT in active cancer patients.

Central venous catheter induced thrombosis

Central venous catheters (CVCs) in the form of surgically tunneled catheters, or totally implanted venous access devices, are increasingly being used for long duration infusion chemotherapy.[50,51] Although these devices have revolutionized the clinical management of cancer patients, they are associated with several complications, including infection, catheter thrombosis and pulmonary embolism (PE). Catheter-related venous thrombosis can lead to considerable morbidity, occasional mortality, and the loss of catheters. The diagnosis of upper extremity deep venous thrombosis (UEDVT) is increasing and constitutes about 18% of all DVTs. About 7–9 % of patients with UEDVT have been reported to develop acute PE.[52] The reported incidence of catheter-related thrombosis varies considerably ranging from 12–60% in various studies.[53] The wide variability in incidence of catheter-related thrombosis is due in part to the differences in catheter type, position, and duration of insertion, type of malignancy and use of different chemotherapeutic agents. Catheter-related thrombosis is frequently under-diagnosed, as most patients are asymptomatic or have non-specific symptoms.

Recurrent venous thromboembolism

Venous thrombosis is often a chronic condition, with recurrence rates estimated at 5% to 7% annually after a first episode.[54,55] The risk is highest among those whose initial episode was associated with cancer, and lowest among those whose initial episode was associated with a temporary risk factor such as surgery.[55] In one study, older age and obesity were associated with higher recurrence risks.[55] Recent reports suggest an approximate 60% higher recurrence risk among men compared to women.[56] The risk appears to be highest in the 6 to 12 months following cessation of anticoagulation, regardless of the initial duration of anticoagulation.[57] In a case–control study, patients with a history of VTE were approximately 8 times more likely to develop a new episode during a subsequent high-risk period compared with patients without a history of DVT or PE.[58] Other risk factors for recurrent thrombosis include residual vein thrombosis on ultrasound, PE as the first thrombosis event, and proximal versus distal limb deep vein thrombosis.

Congestive Heart or Respiratory Failure

Patients with congestive heart or respiratory failure are also at risk of venous thromboembolic complications. In the Prophylaxis in Medical Patients with Enoxaparin (MEDENOX) trial, 15% of patients with class III or IV heart failure treated with placebo had a confirmed episode of VTE.[59] Similarly, 16% of patients with class III or IV heart failure treated with low-dose subcutaneous heparin in the Thromboembolism Prevention in Cardiopulmonary Disease with Enoxaparin (PRINCE) study developed VTE.[60]

Oral Contraceptives

As oral estrogen compounds became widely available in the late 1960s, early reports suggested an alarming incidence of VTE in young and otherwise healthy women taking oral estrogen to prevent conception.[61] Overall risk for thrombosis in oral contraceptive users is about threefold higher than for non-users and is highest during the first year of use.[62,63] Lowering the oestrogen dose reduces risk for thrombosis.[61] Preparations containing third-generation progestogens are associated with an increased risk for thrombosis compared with levonorgestrel.[61] Oral contraceptives enhance thrombosis risk in families with a natural inhibitor deficiency.[64] A 20–30-fold increased risk for thrombosis in heterozygous women with factor V Leiden[65] and a 16-fold enhanced risk in women with the prothrombin mutation[66] have been reported during oral contraceptive intake. However these numbers have to be set into perspective with the very low absolute risk for deep vein thrombosis in young women.[67]

Hormone replacement therapy

In women receiving hormone-replacement therapy (HRT), the estrogen dose is generally 20% to 25% of that contained in modern oral contraceptives. Despite the much lower biological potency, women taking HRT have a 2- to 4-fold increased risk of idiopathic venous thrombosis compared with women not taking HRT.[68,69,70] Women with a history of VTE who are using HRT are at greater risk of recurrence than those with a similar history but not on hormonal therapy.[71] Progestagen is added to estrogen therapy among postmenopausal women with an intact uterus to prevent the elevated risk of estrogen-induced endometrial hyperplasia and adenocarcinoma. Concomitant progestagen use was associated with an increased VTE risk compared with the use of estrogen alone. The

mechanisms underlying the increased VTE risk among users of oral estrogen or transdermal estrogen combined with norpregnane derivatives include a prothrombotic state, a decreased blood flow, and/or an alteration in the vessel wall.[72]

Acquired Lupus Anticoagulants and the Antiphospholipid Syndrome

Antiphospholipid thrombosis syndromes, which include not only the lupus anticoagulant (LA) and anticardiolipin antibodies (ACLAs) but also more recently recognized ‘‘subgroups’’ of antiphospholipid antibodies (antibodies against beta-2-glycoprotein-I [B-2-GP-I]) and antibodies to phosphatidylserine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine, and anti-annexin-V, all comprise the antiphospholipid thrombosis syndromes. Antiphospholipid syndrome is the most common acquired blood protein defect associated with either venous or arterial thrombosis or both. The thrombotic and thrombo-occlusive events associated with these antiphospholipid antibodies include thrombosis of the venous system, the arterial system, coronary artery thrombosis, cerebrovascular thrombosis, transient cerebral ischemic attacks (TIAs), retinal vascular thrombosis, and placental vascular thrombosis (leading to recurrent-miscarriage syndrome).

The antiphospholipid thrombosis syndrome consists of closely related but clearly distinct clinical syndromes that often are discordant with respect to types of antiphospholipid antibodies found; these syndromes are

(1) The LA-thrombosis syndrome,

(2) The ACLA-thrombosis syndrome, and

(3) Thrombosis associated with subgroups of antiphospholipid antibodies.

The ACLA-thrombosis antiphospholipid syndrome is much more common than the LA-thrombosis antiphospholipid syndrome, the ratio being approximately 5:1.

The anticardiolipin syndrome is associated more commonly with both arterial and venous thrombosis, including typical deep vein thrombosis and pulmonary embolus, premature coronary artery disease, premature cerebrovascular disease and retinal arterial and venous occlusive disease. Patients with anticardiolipin thrombosis syndrome develop more predictable types of thrombosis than those with the LA-thrombosis syndrome.

The LA, although sometimes associated with arterial disease, more commonly is associated with venous thrombosis with or without pulmonary embolus.

Patients with thrombosis harboring antibodies to B-2-GP-I or antibodies to other protein epitopes mentioned above tend to more closely resemble patients with ACLAs than patients with isolated LA. Anti-annexin-V antibodies are the newest ‘‘subgroup Antiphospholipid antibody’’ to be noted in association with both arterial and venous thrombosis.

Annexin V, actually a protein bound to phospholipids, is an important vascular component, and antibodies directed against this moiety seem to disrupt endothelium and endothelial function and lead to associated thrombotic tendencies.[72]

Although all of these antiphospholipid antibody–thrombosis syndromes may be seen in association with systemic lupus erythematosus, other connective tissue and autoimmune disorders, and other selected medical conditions such as lymphomas, most individuals (approximately 90%) developing any of the antiphospholipid thrombosis syndromes are otherwise healthy individuals and harbor no other underlying medical condition. These

patients are classified as having primary, rather than secondary, antiphospholipid thrombosis syndrome.[73]

Lupus anticoagulants and thrombosis

In 1952, Conley and Hartmann described a coagulation disorder in two patients with systemic lupus erythematosus; the patients exhibited anticoagulant activity by in vitro testing, which was manifested by a prolonged whole blood clotting time and prothrombin time.[74] Most commonly, the LA develops in otherwise healthy individuals (primary LA-thrombosis syndrome). There is also an association with drug ingestion; commonly associated drugs include chlorpromazine, procainamide, quinidine, hydralazine, Dilantin, interferon, Fansidar, and cocaine.[75]

Patients with the LA are at increased risk for thromboembolic disease, most commonly deep vein thrombosis, pulmonary emboli, and thrombosis of other large vessels.[76] Thromboembolism occurs in approximately 10% of patients with systemic lupus; however, in patients with systemic lupus and the LA, thromboembolism occurs in up to 50% of patients. In patients harboring a primary LA, the LA is estimated to account for approximately 6% to 8% of thrombosis in otherwise healthy individuals.

Although patients also may have arterial events, this is uncommon in primary LA-thrombosis syndrome, as opposed to primary ACLA-thrombosis syndrome, in which arterial events are almost as common as venous events. This observation is in distinction to patients with secondary LA-thrombosis syndrome, wherein patients more commonly experience arterial events than do those with primary LA-thrombosis syndrome. Even in secondary LA-thrombosis syndrome, however, venous events are more common than arterial events. Although the LA is associated with thrombosis, the mechanisms whereby

thrombosis occurs remain unclear. It has been proposed that there might be an interaction with the vasculature, thereby altering prostaglandin release. There may be activation of platelets and changes in prostaglandin metabolism, or the antibodies may block protein C or the activated protein C pathway, or they may alter phospholipids interactions with activated factor V. [77] It also has been proposed that there may be hyperactivity of the fibrinolytic system and increased levels of plasminogen activation inhibitor. Despite many proposed mechanisms, to date there remains no consensus on the precise mechanisms of action of LAC's.[78]

Hemolytic anemia's

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal disorder of bone marrow stem cells. Patients generally manifest chronic intravascular hemolysis, with episodes of gross hemoglobinuria accompanied by leukopenia and thrombocytopenia. Despite the presence of thrombocytopenia, thrombosis is more commonly encountered than bleeding. Indeed, thrombosis can be a presenting feature of PNH, as well as an important cause of morbidity and mortality in this disorder.

A unique feature of PNH is the predilection for thrombosis to occur in the intra-abdominal venous network (mesenteric, hepatic, portal, splenic, and renal veins) and cerebral vessels, as opposed to deep vein thrombosis or pulmonary embolism.[79]

Thrombosis also is a serious complication in patients with other types of hemolytic anemias, including sickle cell disease and thalassemia. In sickle cell disease, increased blood viscosity due to sickled erythrocytes contributes to microvascular occlusion. There is evidence for endothelial activation and increased numbers of circulating microvascular endothelial cells ($CD36^{+}$) over expressing intercellular adhesion molecule-1 (ICAM-1),

vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin, and tissue factor in patients with sickle cell disease.[80] As standards of care for thalassemic patients have improved in recent years, resulting in an almost doubling of life expectancy, previously un-described complications of the disease are being recognized, particularly thromboembolic complications.[81] These include cerebral thrombosis, deep vein thrombosis, pulmonary embolism, and recurrent arterial occlusions. Venous thrombosis develops following splenectomy in some patients. In a Turkish study[82] a total 30 patients (15 female, 15 male) with thalassemia major (n: 16), thalassemia intermedia (n: 11) and S-ß thalassemia (n: 3) were included in the study and the effects of thrombocytosis and natural inhibitors on thrombosis after splenectomy were investigated. Thrombosis was detected in the portal vein system in 7/30 (23.3%) of splenectomized patients. There was no statistical increase of platelet count in patients with or without thrombosis. Natural inhibitor levels in all patients were lower than controls ($p< 0.001$), but there was not any statistical difference between the patients with and without thrombosis.

Myeloproliferative disorder and hyperviscosity

Thrombosis can be a manifestation of diseases associated with hyperviscosity. Hyperviscosity of the blood may be due to increased plasma viscosity, an increased number of red or white blood cells, or decreased deformability of cells.

Hyperviscosity plays an important role in the pathogenesis of thrombosis in polycythemia vera, which is a major complication of this disorder. Patients with elevated hematocrits have increased whole blood viscosity, and this abnormality has been inversely correlated with cerebral blood flow. Common thrombotic complications in this disorder include cerebrovascular accidents, myocardial infarction, peripheral arterial occlusion, deep vein

thrombosis, pulmonary embolism, and portal and hepatic vein thrombosis (Budd-Chiari syndrome).

In the myeloid and monocytic leukemias, the presence of very elevated white blood counts (generally greater than 100,000/mL^3) can increase the viscosity in the microcirculation, which can play a role in the pathogenesis of thrombosis. Small vessels in the lungs, brain, and, less commonly, other organs may be obstructed by high levels of immature leukocytes.

Inherited thrombophilia

The first case of inherited thrombophilia was described in 1965 by Egeberg[73,83] in a publication on a Norwegian family with a remarkable tendency to develop venous thrombosis, which was shown to be due to a deficiency of the physiologic anticoagulant antithrombin. In the 1980s, protein C[84] and protein S[85] deficiencies were recognized. Activated protein C (APC) resistance was identified by Dahlback et al in 1993[86]as a risk factor for venous thrombosis in 1993. In 1994, Bertina et al found that factor V was involved in APC resistance. Subsequently, the factor V Leiden mutation was found by searching the factor V gene of APC resistant patients for mutations in APC binding- and cleavage sites. The second common genetic factor, prothrombin 20210 G/A, was identified in 1996 through screening the prothrombin gene for abnormalities among patients with a personal and family history of venous thrombosis by Poort et al.[87] At present, the three plasma protein deficiencies and the two mutations are the main genetic risk factors for venous thrombosis. However, they still explain only part of venous thrombotic events.[88]

Thus now in >50% of patients with juvenile or idiopathic thrombosis, an inherited thrombophilic condition can now be identified. The predisposing defects do not necessarily cause continuous clinical impairment but they need only weaken the ability to cope with fluctuations induced by interactions with the environment. Patients with thrombophilia can present fulminantly who, without therapy thrombose continuously, but in most patients thrombosis is episodic, separated by often prolonged asymptomatic periods. The discontinuity suggests that there is some trigger for each event, perhaps a direct stimulus or a temporary deterioration of intrinsic resistance, or some combination of these factors, which further enhance the risk. Undoubtedly the concept of inherited thrombophilia is an operational one, the definition of which has undergone continuous refinement. It was originally based upon early presentation of thrombosis, usually coupled with inherited phenotypic abnormality of one of the inhibitory proteins, antithrombin, PC or PS. Progress in the molecular basis of thrombosis has enabled a more genetically based definition to be formulated. Inherited thrombophilia is a genetically determined tendency to venous thromboembolism Dominant abnormalities or combinations of less severe defects may be clinically apparent from early age of onset, frequent recurrences or family history. Instead milder traits maybe discovered only by lab investigations. It is important to emphasize that thrombophilia is even now diagnosed on clinical grounds.[73] Major criteria are listed in Table 2.[73]

Table-2 Most Common Features of Thrombophilia
Recurrent venous thromboembolism
Thrombosis in an unusual site (cerebral sinuses, mesenteric, portal)
Venous thromboembolism at a young age
Family history of venous thromboembolism
Recurrent fetal loss
Pre-eclampsia, HELLP syndrome

Inherited thrombophilic disorders (Table-3) can be divided into loss-of-coagulation function disorders and gain-of-coagulation function disorders.[89] Loss-of-function disorders include deficiencies of the endogenous anticoagulants antithrombin, protein C, and protein S. They are less common than gain-of-function disorders but may be more potent risk factors for thrombosis. Gain-of-function disorders include factor V Leiden, the prothrombin G20210A variant, and possibly elevation of procoagulant factors such as factor VIII, von Willebrand factor, and factors V, VII, IX, and XI. These may be weaker risk factors for venous thromboembolism in general.

Table 3- Inherited thrombophilia
Natural inhibitor deficiency • Protein C • Protein S • Anti-thrombin
Hyperhomocysteinaemia /MTHFR mutations
Prothrombin G20210A
APC resistance (Factor V Leiden)
High factor VIII or factor IX
Dysfibrinogenaemia or hyperfibrinogenaemia
Antiphospholipid antibody syndrome
Fibrinolytic pathway defect
Others

Blood coagulation system

The current concept of blood coagulation is that it is initiated by small amounts of encrypted TF which circulate in blood bound to microparticles comprising small phospholipids vesicles with bound proteins. On local enrichment of these particles, the

concentration of TF exceeds a certain threshold needed to trigger coagulation. This combines with small amounts of activated factor VII (VIIa) in the circulation to form TF:VIIa complexes. The FVIIa-TF complex directly activates Factor X and generates Xa, which along with FVa activates prothrombin and converts it to small amounts of thrombin. This small amount of thrombin generated at sites of vascular injury expresses several procoagulant properties. In a positive feedback reaction, it converts the procofactors FV and FVIII into their biologically active forms FVa and FVIIIa.[90] More recently, it has become apparent that thrombin also plays a positive feedback role through the activation of FXI.[91] Small amounts of FXIa catalyze the formation of FIXa, which in turn activates FX, thus enforcing thrombin formation. In addition, thrombin activates platelets and ultimately converts fibrinogen into fibrin.[92] Further functions of thrombin, aimed at the generation of a stable fibrin plug, comprise the activation of the transglutaminase FXIII into its activated form and the activation of the thrombin-activatable fibrinolysis inhibitor (TAFI). These processes stabilize the fibrin clot, FXIIIa, by cross-linkage of fibrin monomers and TAFI through the inhibition of fibrinolysis.[93]

Protein C system

The highly efficient reactions of the coagulation system have considerable biological potential, and strict regulation is therefore required. To this end, several plasma proteins and multiple cell-cell and cell-matrix interactions are involved in the continuous monitoring of the circulation. At each level of the coagulation pathway, membrane-bound molecules expressed on the surface of intact endothelial cells, circulating inhibitors, or negative feedback mechanisms provide efficient control.[94,95] Similar processes of limited proteolyses that govern the formation of procoagulant enzymes are also involved in the

down-regulation of the activities of activated clotting factors. Most strikingly, thrombin itself is the key factor to the initiation of a natural anticoagulant pathway called the protein C anticoagulant system.

A key event at the initiation of the protein C pathway is when thrombin alters its substrate specificity and changes from a procoagulant to an anticoagulant protein. This change is dependent on the presence of thrombomodulin (TM), a transmembrane thrombin receptor present on intact endothelium. Thrombin bound to TM has lost its procoagulant properties but rapidly activates protein C in a process that is enhanced by the endothelial cell protein C receptor (EPCR).[96] Protein C is a vitamin K-dependent protein circulating in plasma at a concentration of 50 to 80 nM.[97] During activation of protein C by the thrombin-TM-EPCR complex, a 12-amino acid activation peptide is released, and the activated protein C exhibits a highly specific proteolytic activity, cleaving only a limited number of peptide bonds in FV/FVa and FVIIIa.[98]

Protein C deficiency

Deficiencies of protein C may be either congenital or acquired.[99] Congenital deficiency is autosomal dominant and characterized by recurrent venous thrombosis and thromboembolism beginning in adolescence.[92] Most homozygous patients die of thromboembolic disease in infancy.[92] Both absence and dysfunctional forms of the disorder are observed. Type I disease is characterized by reduction in both antigenic and functional levels to less than 50% and type II, in which functional levels are decreased much more than antigenic levels.[92] Studies of the genetic defects in patients with protein C deficiency have led to the identification of more than 160 different mutations.[100] Heterozygous protein C deficiency is inherited in an autosomal dominant fashion. The

phenotype of patients with heterozygous protein C deficiency is similar to that of persons with hereditary antithrombin III deficiency. In severely affected families, approximately 75% of protein C-deficient persons experienced one or more thrombotic events. The initial episode occurs apparently spontaneously in approximately 70% of those experiencing such events. The remaining 30% have the usual associated risk factors (pregnancy, parturition, contraceptive pill use, surgery, or trauma) at the time they develop acute thrombotic events.

The most common sites of disease are the deep veins of the legs, the iliofemoral veins, and the mesenteric veins. Approximately 63% of affected patients develop recurrent venous thrombosis, and approximately 40% exhibit signs of pulmonary embolism.[101] Superficial thrombophlebitis of the leg veins as well as cerebral venous thrombosis can occur in protein C-deficient patients.[102,103]

The prevalence of protein C deficiency in outpatients presenting with an initial episode of venous thromboembolism ranges from 0.5% to 4%.[104,105,106] In earlier reports of more selected patient populations, protein C deficiency was more frequently identified, ranging from 2% to 9%.[107,108] In the general population, the prevalence of protein C deficiency is between 1:200 and 1:500, as documented by mass screening of blood donors.[109,110]

Protein S system

Protein S, an important cofactor to APC, is also a vitamin K-dependent plasma protein, circulating in plasma. In human plasma, approximately 40% of protein S is free, and the remaining 60% is bound to the complement regulatory C4b-binding protein (C4BP). Only the free form functions as APC cofactor.

The mechanism by which free protein S acts as a cofactor to APC is not yet fully understood. It has been suggested that protein S exerts its APC cofactor activity through enhancement of the binding of APC to phospholipid surfaces. In the absence of protein S, inactivation of membrane bound FVa proceeds primarily through the cleavage of two peptide bonds at Arg 506 and Arg 306. Cleavage at Arg 506 is kinetically preferred over that at Arg 306, showing an approximate 20-fold difference in overall reaction rate. However FXa has been shown to protect the Arg506 site. Moreover, cleavage at Arg506 does not fully abrogate FVa activity, and complete loss of FVa activity requires a subsequent (slower) cleavage at Arg306. Protein S specifically promotes the cleavage at Arg306. Thus, in the presence of both FXa and protein S, though the preferred cleavage at Arg506 is blocked, FVa is readily inactivated through the cleavage at Arg306.[111]

Protein S deficiency

In 1984, members from several kindreds who exhibited reduced levels of protein S were described who had a striking history of recurrent venous thrombotic disease.[112] Subsequently, many additional families with this disorder have been reported.

Heterozygous protein S deficiency is inherited in an autosomal dominant fashion, with a reported frequency of approximately 10% in families with inherited thrombophilia.[113] However, the prevalence is less (between 1% and 7%) among consecutive outpatients with deep vein thrombosis.[97,98] Protein S deficiency generally is considered to confer a risk of thrombosis similar to that in protein C deficiency.[114,115]

The clinical presentation of patients with heterozygous protein S deficiency is similar to that outlined for deficiencies of antithrombin III and protein C. Of 71 protein S-deficient members from 12 Dutch pedigrees,[116] 74%, 72%, and 38% sustained deep vein

thrombosis, superficial thrombophlebitis, and pulmonary emboli, respectively. The mean age at the first thrombotic event was 28 years, with a range of 15 to 68; 56% of the episodes apparently were spontaneous, and the remainder were precipitated by an identifiable factor. Thrombosis also has been reported in the axillary, mesenteric, and cerebral veins.

Three types of protein S deficiency states can be identified on the basis of measurements of total and free antigen as well as functional activity (Table-4). Type I deficiency is associated with approximately 50% of the normal total protein S antigen level[117] and decrements in free protein S antigen and protein S functional activity to less than approximately 40% of normal.[118] Another type of hereditary deficiency (type II) has been described in which the functional activity of protein S is decreased, but with normal total and free antigen levels. A type III deficiency state is characterized phenotypically by a decreased concentration of free and functional plasma protein S but a normal level of total protein S antigen.

Table 4 Assay Measurements in Heterozygous Protein S Deficiency

Type	Protein S Total	Antigen Free	Protein S Activity
I ("classic")	Low	Low	Low
II	Normal	Normal	Low
III	Normal	Low	Low

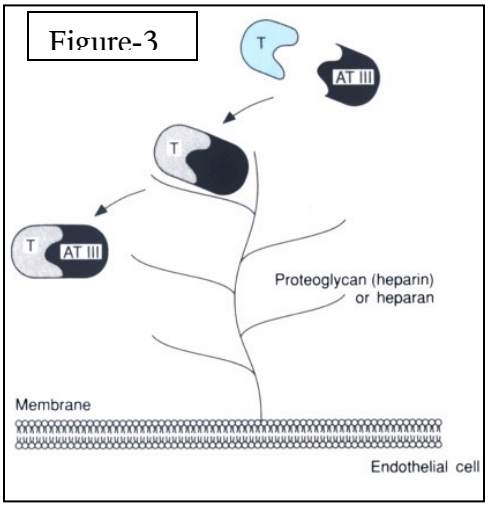
Type I deficiency most often is secondary to missense mutations, base pair insertions or deletions, premature stop codons, or mutations affecting a splice site. There have been a few cases of large deletions of the gene. Of the more than 130 different mutations documented in patients with various types of protein S deficiency, only 7 different nucleotide substitutions have been identified with a type II deficiency.[119] Several of these

mutations are located in the N-terminal end of the protein S molecule, which includes the domains that interact with activated protein C.[120] The biologic basis of type III protein S deficiency state is uncertain.

Antithrombin

The concept of antithrombin stems from P. Morawitz in 1905 who considered it responsible for the gradual loss of thrombin activity after blood had clotted.[121] Antithrombin is a glycoprotein inhibitor that functions by neutralizing coagulation proteases (fig-3), by forming a 1:1 complex between the enzyme and antithrombin (AT). This reaction probably accounts for the major mechanism of inhibition of thrombin and factors Xa and XIa, but the reaction is slow in the absence of heparin. Heparin accelerates inhibition of the coagulation proteases about 1000-fold. As a result, antithrombin must be present for heparin to function as an effective anticoagulant. With thrombin, optimal rates of inactivation require the simultaneous interaction of heparin with antithrombin and thrombin.[122] Heparin acts as a bridge between thrombin and antithrombin, thereby concentrating the reactants and accelerating the reaction. In addition, the interaction of heparin with antithrombin alters the conformation of the inhibitor, which makes the protease "trap" more accessible to thrombin and the other coagulation factors.[115] The vascular endothelium and the microvascular endothelium in particular, are rich in anticoagulantly active heparin-like proteoglycans, which are endothelial cell-associated proteins with heparin side chains bearing the critical carbohydrate sequences required for antithrombin recognition. Both thrombin and antithrombin bind to sulfated carbohydrates and are brought into close proximity to each other. Antithrombin undergoes a conformational change required for rapid inactivation of thrombin. Heparin functions

catalytically, facilitating the rapid dissociation of the thrombin/antithrombin complex from the proteoglycan. This critical step is responsible for re-exposure of the heparin sites for binding with other thrombin and antithrombin molecules.



Antithrombin III Deficiency

Antithrombin III deficiency is inherited in an autosomal dominant fashion and thus affects both genders equally. Two major types of inherited antithrombin III deficiency have been delineated. The type I deficiency state is a result of reduced synthesis of biologically normal protease inhibitor molecules.[123] In these cases, the antigenic and functional activity of antithrombin III in the blood is reduced in parallel. The molecular basis of this disorder is either a deletion of a major segment of the antithrombin III gene or, more commonly, the occurrence of small deletions/insertions, or single base substitutions. These mutations will introduce a frameshift, a direct termination codon, a change in messenger RNA (mRNA) processing, or unstable translation products. The antithrombin III mutation database includes 80 distinct mutations in patients with a type I deficiency.[124] The second type of antithrombin III deficiency is produced by a discrete molecular defect within the protease inhibitor (type II). The plasma levels of

antithrombin III are greatly reduced as judged by functional activity, whereas antithrombin III immunologic activity is essentially normal. The prevalence of antithrombin III deficiency in the adult population is approximately 1/2000.[125] Studies of healthy blood donors employing functional assays that measure heparin cofactor activity have found that the prevalence of antithrombin III deficiency in the general population is 1/250 to 1/500.

The initial clinical manifestations occur apparently spontaneously in about 42% of persons but are related to pregnancy, parturition, use of oral contraceptives, surgery, or trauma in the remaining 58% of patients.[126] The most common sites of disease are the deep veins of the leg and the mesenteric veins. Recurrent thrombotic episodes occur in approximately 60% of affected persons, and clinical signs of pulmonary embolism are evident in 40%.[119] Thrombotic episodes are rare in affected children before puberty. At this time, thrombotic events start to occur with some frequency, and the risk of thrombosis increases substantially with advancing age.[119] First-degree relatives of symptomatic persons with antithrombin III deficiency have an 8- to 10-fold increased risk of thrombosis over that in noncarriers.[106] In the Leiden Thrombophilia Study, a case-control study of 474 consecutive patients following an initial episode of deep vein thrombosis,[127] the prevalence of antithrombin III deficiency was only 1.1%, and the odds ratio for thrombosis was only 5.0.[98]

Factor V – Physiology

FV is a large single chain glycoprotein of approximately 330 kDa. Even though several cellular types have been reported to synthesize FV, the principal site of biosynthesis remains the liver. Human FV is synthesized as a single-chain molecule that undergoes

extensive posttranslational modifications before it is secreted into the blood. FV has a mosaic-like structure, with a domain organization A1-A2-B-A3-C1-C2 (Fig. 4) that is similar to that of FVIII.[128]

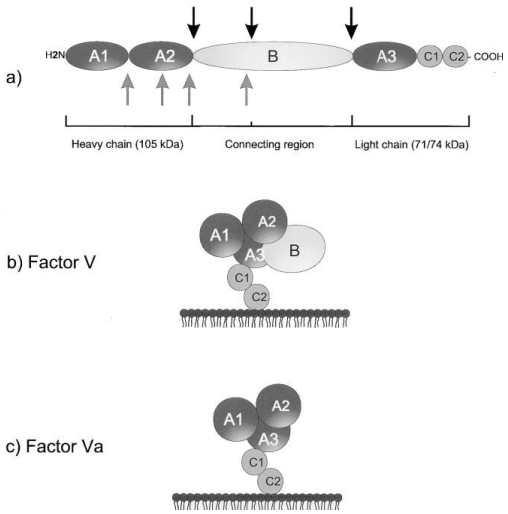


Figure-4 Domain structure of factors V. The large B domain separates the A2 and A3 domains. The three A domains are arranged in a triangular fashion, with the B domain extending from the A2 and A3 domains (b). Thrombin-mediated cleavages at Arg709, Arg1018, and Arg1545 (A, upper black arrows) result in B-domain liberation and activation of FV. Active FVa comprises the A1-A2 heavy chain noncovalently bound to the A3-C1-C2 light chain, and the C2 domain localizes the protein to negatively charged phospholipid membranes (c). Proteolytic inactivation of FV(a) catalyzed by APC proceeds through peptide bond cleavages at Arg306, Arg506, Arg679, and Lys994, as indicated by the lower arrows (a).[130]

Circulating single-chain FV is an inactive procofactor with the potential to express either procoagulant or anticoagulant activities after proteolytic modification by procoagulant or anticoagulant enzymes (Fig. 4). Cleavage of FV by either FXa or thrombin results in the conversion of FV to the procoagulant cofactor FVa, whereas APC-mediated cleavage of intact FV converts the molecule to an anticoagulant species with the capacity to support APC in the degradation of FVIIIa. (Fig-5) The APC-cofactor activity of FV and that of protein S are synergistic, and only in the presence of both proteins is the FVIIIa in the tenase complex efficiently inhibited by APC.[129,130]

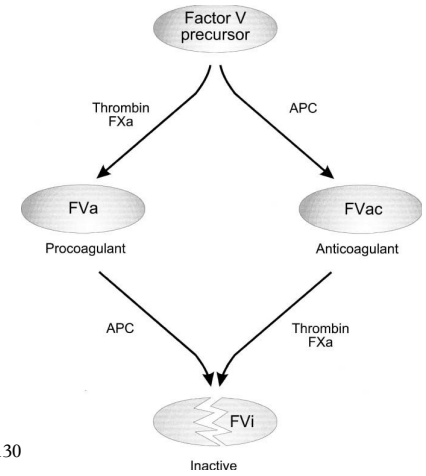


Figure-5[130]

The dual functions of FV suggest that it can act as a local sensor of procoagulant and anticoagulant forces. In the event the procoagulant enzymes FXa and thrombin are generated, FV is converted to a procoagulant FXa cofactor. In contrast, if APC predominates, FV is converted to an anticoagulant molecule functioning as APC cofactor. Circulating single-chain FV expresses less than 1% of the procoagulant FXa-cofactor activity it can maximally obtain. Activation of FV by procoagulant enzymes such as thrombin and FXa comprises the limited proteolysis of three peptide bonds at Arg709 (between A2 and B), Arg1018 and Arg1545 (between B and A3), resulting in an increase of the FXa-cofactor activity.[131] These cleavages result in dissociation of the large connecting B domain (Fig. 4) from FVa. APC cleaves FVa at Arg306, Arg506, and Arg679. As a consequence of these cleavages, the peptides generated dissociate from each other, thereby inducing a loss of cofactor activity. In the degradation of FVIIIa, the activity of APC is enhanced several-fold by the presence of the protein cofactors protein S and intact FV.[132] It has been observed that full activation of FV—that is, after cleavage at Arg709 and Arg1545, resulting in the release of the B domain, leads to a loss of anticoagulant activity of FV.[122]

FV Leiden-induced APC resistance

In 1993, Dahlbäck[133] identified a novel mechanism for familial thrombophilia; the plasmas of persons with unexplained personal and familial histories of venous thromboembolism exhibited a poor response to activated protein C (APC) in a PTT assay. The observations of Dahlbäck facilitated the development of a PTT-based assay to screen for APC resistance. In a U.S. referral population of patients younger than 50 years of age with unexplained venous thromboembolic disease, approximately 50% were found to have APC resistance.[134] Investigators in Italy and Austria confirmed that APC resistance was a frequent laboratory abnormality in patients with unexplained venous thrombosis.[135]

In 1987, Dutch investigators from Leiden initiated the Leiden Thrombophilia Study, a large case-control study to investigate risk factors for first episodes of venous thrombosis in the general population of The Netherlands.[136] It was shown that a defect in factor V involving the mutation -Arg506Gln, or factor V Leiden is most often the cause of APC resistance.[137] This is the site at which APC cleaves factor Va, and this sequence alteration makes the mutant factor Va molecule biochemically resistant to inactivation by the enzyme.[130,138] The Arg506Gln substitution was found to be the cause of APC resistance in about 90% of Dutch patients with APC resistance on the PTT assay, and the mutation was found in 2% to 4% of healthy Dutch controls. Most patients with APC resistance are heterozygous for the factor V Leiden mutation, but a number of homozygous patients with heightened APC resistance on PTT assays have been identified.[130] Homozygotes are at higher thrombotic risk than that noted for heterozygotes.[139]

FVLeiden and thrombosis

The estimated risk for thrombosis from the presence of the FVLeiden mutation is similar to that of other genetic defects affecting the protein C anticoagulant pathway. The relative risk in heterozygous carriers of the FVLeiden mutation is increased 5- to 7-fold, whereas it is increased up to 80-fold for homozygous persons. Thromboembolic episodes associated with FVLeiden are almost exclusively venous in nature. The severity and localization of thrombosis in carriers of FVLeiden is diverse. Common are thromboses in the deep veins of the leg,[140] whereas portal vein thrombosis, superficial vein thrombosis, and cerebral vein thrombosis are less prevalent. However, primary pulmonary embolism[141] does not seem to be associated with the FVLeiden mutation. Combinations of other thrombotic risk factors, either genetic or acquired, and FVLeiden appear to have a synergistic effect. Factor V Leiden and as well as other hereditary thrombophilia the prothrombin gene mutation are associated with an approximate tripling of the risk of late fetal loss.[142,143] An increased incidence of factor V Leiden, as well as other thrombophilias, also was reported in women in association with other obstetric complications.[144]

The prevalence of heterozygosity for the factor V Leiden mutation in whites, including European, Jewish, Israeli Arab, and Indian populations, ranges between 1% and 8.5%.[145]

APC resistance in the absence of the FV Leiden mutation

Already in the earliest reports on APC resistance, it was noted that there was no 100% association between the presence of the FV Leiden mutation and APC resistance.[130] In approximately 5% of all patients with phenotypic APC resistance, the Arg506Gln was absent. Two mutations at the Arg306 residue in factor V, the second APC cleavage site in

the activated cofactor, have been described in patients with a history of thrombosis. These mutations are replacement of Arg306 with threonine (factor V Cambridge)[146] and with glycine (in Hong Kong Chinese).[147] For both FV forms, the observed APC resistance phenotype was intermediate between normal FV and FV Leiden.

Prothrombin G20210A mutation

The prothrombin G20210A mutation in the 3' UTR was first described by Poort et al in 1996[78] and results in elevated plasma prothrombin levels and an increased risk of venous thrombosis.[148] This mutation is located at the last position of the 3'-untranslated region at the cleavage site for polyadenylation of prothrombin mRNA changes the position of the 3'-cleavage/polyadenylation reaction in prothrombin mRNA, thereby leading to increased prothrombin biosynthesis by the liver.[149] The gene mutation now is known to be associated with both arterial and venous thrombosis. Subsequently, Brown et al[150] reported a study of 504 patients with venous thromboembolism, and 2.6% of this population was positive for the prothrombin G20210A gene mutation. Prothrombin gene mutation, similar to the factor V Leiden mutation, seems to be rare in individuals of African and Asian decent. In the Leiden Thrombophilia Study, 6.2% of venous thrombosis patients and 2.3% of healthy matched controls had the prothrombin gene mutation.[139]

Hyperhomocysteinemia and thrombosis

Homocysteine metabolism[151] - Remethylation and transsulfuration

Homocysteine is an intermediary in the methionine cycle (Fig.6). Methionine derived from dietary sources is first converted into S-adenosylmethionine (SAM). This important

intermediate serves as the key methyl donor for a large number of biochemical reactions. After donating a methyl group, SAM is converted to S-adenosylhomocysteine, which undergoes hydrolysis to form adenosine and homocysteine.

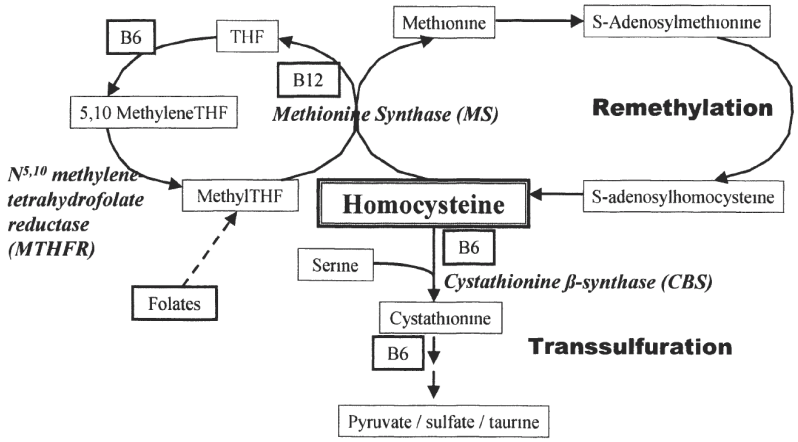


Figure-6[153]

Homocysteine is remethylated to form methionine by one of two pathways. The major pathway is remethylation by methionine synthase (MS) in a reaction that requires vitamin B12 as a cofactor and converts N5-methyltetrahydrofolate (MTHF) to tetrahydrofolate (THF). THF is recycled into MTHF in a two-step process; the first step converts THF to N [5],N10- methylenetetrahydrofolate, and the second converts N [5],N10-methylenetetrahydrofolate back to 5-methylTHF. This second step is catalyzed by methyltetrahydrofolate reductase, an enzyme under much study because of a common mutation that produces mild hyperhomocysteinemia. The minor homocysteine remethylation pathway that uses betaine as a methyl donor becomes important clinically in the treatment of patients with homocystinuria. The methionine cycle and the linked 5-methylTHF regeneration cycle form the primary pathway of homocysteine metabolism known as the remethylation pathway.

Alternatively, homocysteine can be metabolized by the transsulfuration pathway. Homocysteine can condense with serine to form cystathionine by the action of

cystathionine b-synthase (CBS). A congenital deficiency of this enzyme causes most of the homocystinuria cases associated with early-onset atherosclerosis.

MTHFR mutations: Cys677Thr

Mutations that severely reduce MTHFR activity are rare. In 1988, Kang et al[152] reported a thermolabile mutation of MTHFR associated with less specific enzyme activity and hyperhomocysteinemia. In 1994 the MTHFR gene was cloned, and, shortly thereafter, Frosst et al[153] identified the common polymorphism of MTHFR (Cys677Thr) that showed thermolability and produced hyperhomocysteinemia. This point mutation replaces an alanine with a valine residue in the enzyme.

It is now known that the Cys677Thr mutant allele occurs at high frequency in Europe (up to 50%)[154] and variable frequency in Asia (approximately 45% in Koreans, 30% in Chinese).[155,156] Given the high T-allele frequency in many populations, heterozygosity and homozygosity are common. It has been consistently shown that heterozygotes have no significant levels of hyperhomocysteinemia, and homozygotes (TT genotype) have raised tHcy values by an average of 25%. [157,158]

Homocysteine is able to promote vascular smooth muscle cell proliferation and inhibit endothelial cell growth.[159] Moderate hyperhomocysteinemia induced by dietary manipulation leads to vascular dysfunction with impaired vasomotor responses, as well as decreased thrombomodulin activity in the aorta. The thrombogenic effects of hyperhomocysteinemia that have been demonstrated in vitro include induction of tissue factor expression,[160] inhibition of heparan sulfate expression, inhibition of nitric oxide and prostacyclin release, inhibition of tissue-type plasminogen activator binding, and inhibition of thrombomodulin-dependent protein C activation.[161]

A relationship between hyperhomocysteinemia and venous thrombosis is now appreciated. Studies of patients who have an initial episode of venous thrombosis before age 45 demonstrate that 13% to 18% have moderate hyperhomocysteinemia.[162] A Dutch study showed that 25% of patients with recurrent venous thrombosis had homocysteine levels above the 90th precentile of those in healthy controls, and the presence of this abnormality conferred a two-fold increased risk of recurrence.[163] In the Leiden Thrombophilia Study, an elevated homocysteine level exceeding the 95th percentile of the control group was found in 10% of patients and conferred a twofold increased risk of thrombosis. The risk of thrombosis was increased 20-fold in patients with idiopathic thrombosis who were both hyperhomocysteinemic and had the Factor V Leiden mutation.

Other plasma coagulation Factors

Factor VIII

Activated factor VIII (FVIII) is the cofactor for activated factor IX (FIX) in the activation of factor X (FX). In 1995, Koster et al studied the influence of ABO blood group, levels of VWF and levels of FVIII on the risk of a first venous thrombosis in the first 301 patients and 301 controls of the LETS.[164] In individuals with FVIII levels over 150 IU/dL the risk of thrombosis increased nearly fivefold compared to individuals with levels lower than 100 IU/dL. The results from the LETS have been confirmed in a number of other independent studies.[165,166] Besides increasing the risk of a first venous thrombosis, high levels of FVIII also may increase the risk of recurrences.[167] The mechanism through which high FVIII levels could influence thrombosis risk has not been fully clarified. It has been reported that high levels of FVIII associate with decreased APC sensitivity.[168] High levels of FVIII can drastically increase thrombin generation.[169]

Factor IX

In 2000, Van Hylckama Vlieg et al reported that individuals with FIX levels over the 90th percentile (>129 U/dL) have a nearly threefold increase in thrombosis risk when compared to individuals with levels below this cutoff value in the LETS.[170] The risk remained after adjustment for possible confounders. The risk of thrombosis appeared to increase linearly with FIX levels. The authors reported that the risk was slightly higher in women than in men and that the risk was highest in premenopausal women not using oral contraceptives. Similar findings were found in a study consisting of 66 women with venous thromboembolism (VTE) and 163 controls between the ages of 45 and 64 years.[171] After adjustment for hormone replacement therapy, women with high FIX levels (>150 U/dL) had a 2.3-fold increase in the risk of VTE. In an Austrian study, it was found that high levels of FIX also increase the risk of recurrence after a previous thrombosis.[172] High levels of FIX increased the risk of recurrence caused by high levels of FVIII from 2.7 to 6.6.

Hyperfibrinogenemia/ Dysfibrinogenemia

In 1994, Koster et al[173] first described the association between high levels of fibrinogen and the risk of a first venous thrombosis. In 199 patients and 199 healthy control subjects from the Leiden Thrombophilia Study (LETS), individuals with fibrinogen levels over 5 g/L had a nearly fourfold increase in risk of venous thrombosis. Furthermore, there was a dose-response relationship between levels of fibrinogen and the risk of thrombosis. A 2003 reanalysis of the LETS included 474 patients and 474 healthy controls.[174] Again, an increase in the risk of thrombosis was observed for high levels of fibrinogen.

Qualitative abnormalities of fibrinogen, inherited in an autosomal dominant manner, are a heterogeneous group of disorders that may be asymptomatic or manifest with a bleeding diathesis or recurrent venous or arterial thromboembolism. A small number of variant fibrinogens have been reported to be associated with thrombotic complications. The functional and biochemical defects of a number of abnormal fibrinogens associated with thromboembolic disease have been characterized.[175] The conversion of fibrinogen to fibrin by thrombin results in the proteolytic cleavage of fibrinopeptides A and B from the molecule. Defects in the release of these two peptides or abnormalities in fibrin polymerization have been reported. In one of the affected kindreds, three homozygous siblings with a Bβ chain substitution of Ala by Thr at position 68 had a severely affected clinical phenotype and sustained both arterial and venous thrombosis at a young age.[176] Decreased binding of thrombin by this mutant fibrinogen has been suggested to lead to the presence of excessive thrombin in the circulation and the occurrence of thrombosis. Other fibrinogen mutants have been shown to cause abnormal fibrin polymerization. Some of the abnormal fibrinogens have been evaluated for their ability to resist or promote fibrinolysis on incorporation into a fibrin clot.

DESIGN AND OBJECTIVES

Objectives of the study

- To study the clinical and laboratory profile of patients with documented thrombosis, which includes- Deep vein thrombosis, pulmonary embolism, cortical sinus thrombosis and Budd Chiari syndrome due to thrombosis of hepatic or Inferior vena caval venous system, who presented to the Christian Medical College and Hospital from January 2005 till June 2007.
- To study the incidence of inherited thrombophilias in patients with documented thrombosis.

Design of the study

This study is a retrospective analysis of patients with venous thromboembolism. The case records and the laboratory data of patients with documented venous thromboembolism were analyzed. The diagnosis of thrombosis was based on radiological evaluation- by Colour doppler ultrasonography in cases of deep vein thrombosis and CT angiogram, Magnetic resonance imaging and angiogram , where indicated.

PATIENTS AND METHODS

Patients who were referred for evaluation of risk factors for venous thromboembolism from January 01, 2005 to June 30, 2007 were initially taken. From this group, the patients who had documented thrombosis in our institution were included for this study. Clinical details, including date of diagnosis of thrombosis, site of thrombosis, family history, recurrence and details of anticoagulation were obtained from the patients' records. The thrombophilia risk workup comprises the following tests:

Complete blood counts

Plasma coagulation tests

- Prothrombin time
- Activated partial thromboplastin time
- Thrombin time

Factor VIII assay

Fibrinogen assay

Natural inhibitors of coagulation

- Protein C assay
- Protein S assay
- Antithrombin assay

Activated protein C resistance

PNH workup

- Ham's and sucrose lysis test

Sickle preparation

Antiphospholipid antibody syndrome

- Dilute Russell's viper venom test (DRVVT)

DNA mutations predisposing for thrombosis, comprising of Factor V Leiden (Arg506Gln), Prothrombin (G20210A) and MTHFR (Cys677Thr) mutations were done in the Hematology department, whenever requested for.

RESULTS

Between the year January 2005 and June 2007, a total of 266 patients with documented thrombosis were evaluated for risk factors of thrombosis in our institution. The median age of presentation was 38 years (range: 4-74). 4 (1.5%) were 4-15 years old, 183 (68.8%) were between 16-45 years and 79 (29.7%) were between 46-74 years (Table 1). There were 140 (52.6%) males and 126 (47.4%) females in our group. This study group contained 38 (14.3%) patients with history of recurrent thrombotic events and only 10 (3.8%) with a positive family history of thrombosis (Table 2). None of the 4 patients in the less than 15 years group had either family history or history of recurrent episodes. 21/183 (11.5%) and 17/79 (21.5%) patients had history of recurrence and 7/183 (3.8%) and 3/79 (3.8%) patients had a positive family history in the 16-45 and 46-74 years age groups respectively.

Table 1: Age distribution

Age (yrs)	Number	%
4-15	4	1.5
16-45	183	68.8
46-74	79	29.7
Total	266	100

Table 2: History of recurrence and family history

	Recurrence history		Family history	
	Number	**%**	**Number**	**%**
Present	38	14.3	10	3.8
Absent	228	85.7	256	96.2
Total	**266**	**100**	**266**	**100**

Thrombosis in the deep veins of the limbs were the most common site of thrombosis in this group, seen in 123 (46.2%) of patients, followed by cortical sinus thrombosis in 92 (34.6%) of patients. Pulmonary embolism as the presenting complains was seen in 26 (9.8%) patients and Budd Chiari syndrome with thrombosis of the hepatic veins or inferior vena cava in 20 (7.5%) patients (Table 3).

Table 3: Site of thrombosis at presentation

Site	Number of patients	%
Deep vein thrombosis (DVT)	123	46.2
Cortical sinus thrombosis (CST)	92	34.6
Pulmonary embolism (PE)	26	9.8
Budd Chiari syndrome (BCS)	20	7.5
CVT + DVT	4	1.5
DVT + PE	1	0.4
Total	**266**	**100**

Among patients with deep vein thrombosis, proximal lower limb veins were affected in 82 (64.1%), followed by abdominal veins in 32 (25%) patients. Distal veins of the lower limb, upper limb veins, veins of the neck or chest or extensive thrombosis, involving more than 2 sites were seen in 4 (3.2%), 5 (3.8%), 4 (3.2%) and 1 (0.7%) respectively. Patients with Budd Chiari syndrome had thrombosis of the hepatic vein in 15 (75%), Inferior vena cava in 2 (10%) and both in 3 (15%) patients.

In our group 121 (45.5%) patients were on anticoagulant therapy. Of these 79 (65.3%) patients were on oral anticoagulation and 42 (34.7%) were on parenteral (Low molecular weight or un-fractionated heparin) Table 4.

Table 4: Patients on anticoagulation

Anticoagulation		Number		%	
Oral		79		65.3	
Parenteral	**LMWH**	42	24	34.7	19.8
	UFH		18		14.9
Total		**121**		**100**	

Acquired etiological factors of thrombosis.

Etiological factors contributing for development of thrombosis were present in 55/266 (20.7%) patients. The breakdown of the etiological factors between the three groups- Venous thromboembolism (VTE), cortical sinus thrombosis (CST) and Budd Chiari syndrome (BCS) is given in Table 5.

30/154 (19.5%) patients with venous thromboembolism, 19/92 (20.6%) patients with cortical sinus thrombosis and 6/20 (30%) patients with Budd chiari syndrome had documented etiological factors as detailed below.

Table 5: Acquired etiological factors

Etiological factors	Whole group (n-266)	VTE (n-154)	CST (n-92)	BCS (n-20)
Lupus anticoagulant	21/266 (7.9%)	17/154 (11.0%)	3/92 (3.2%)	1/20 (5%)
Malignancies				
Acute lymphoid leukemia	2/266 (0.7%)	-	2/92 (2.2%)	-
Acute promyelocytic leukemia	1/266 (0.4%)	1/154 (0.6%)	-	-
Myelofibrosis	1/266 (0.7%)	-	1/92 (1.1%)	-
Polycythemia	5/266 (1.9%)	4/154 (2.4%)	1/92 (1.1%)	-
Lymphoma	1/266 (0.4%)	1/154 (0.6%)	-	-
Others				
Post surgery	4/266 (1.4%)	2/154 (1.2%)	-	2/20 (10%)
Post partum	12/266 (4.4%)	1/154 (0.6%)	11(11.9%)	-
Oral contraceptives	2/266 (0.7%)	-	-	2/20 (10%)
Tb Meningitis	1/266 (0.4%)	-	1/92 (1.1%)	-
Rheumatoid arthritis	1/266 (0.4%)	1/154 (0.6%)	-	-
Chronic liver disease	2/266 (0.7%)	1/154 (0.6%)	-	1/20 (5%)
Pancreatitis	2/266 (0.7%)	2/154 (1.2%)	-	-
Total	**55/266 (20.7%)**	**30/154(19.5%)**	**19/92(20.6%)**	**6/20(30%)**

Blood counts

The median hemoglobin of our whole group was 12 gm% (5.2 – 19.5), total white cell count was 8200/cmm (1300-57800), and platelet count 274000/cmm (10,000-12, 74,000).

Plasma coagulation tests

The median prothrombin time of patients in this study group was 12.6 (8.9-104.3) sec, activated partial thromboplastin time was 30.4 (20.1-166.5) sec and thrombin time was 13.9 (10.7-32) sec. Activated partial thromboplastin time was shortened below the normal range in 13 (4.9%) of patients.

Plasma coagulation factors

Plasma coagulation factors evaluated in this group of patients were factor VIII and fibrinogen. The mean factor VIII level in this study group was 305% (74-725) and fibrinogen level was 318 mg% (156-649). Hyperfibrinogenemia with levels of fibrinogen more than 450 mg% was seen in 28 (10.5%) patients. Fibrinogen levels were elevated in

1/4 (25%) patient, 20/183 (10.9%) patients and 7/79 (8.9%) patients in the age groups 4-15 years, 16-45 years and 46-74 years respectively. Since two studies in the past have demonstrated significant risk of thrombosis and recurrence of thrombosis if factor VIII levels are more than 250 Iu/dL[177] or 294 Iu/dL[178], the cut off for significant elevation of factor VIII levels was taken as > 250 Iu/dL. Thus 159/266 (59.7%) of patients had elevated factor VIII levels. 48/266 (18%) patients had levels more than 450%, 41/266 (15.4%) had levels ranging between 351-450%, 70 (26.3%) patients had levels between 251-350%. (Table 6)

Table 6: Elevated factor VIII levels

Factor VIII	**No**	**%**
251-350	70/266	26.3
351-450	41/266	15.4
>450	48/266	18.0
Total	**159/266**	**59.7**

Natural inhibitors of coagulation

Protein C and S system

The median protein C level in this group was 96 IU/ml (10-287) and that of protein S was 79 IU/ml (12-778). Protein C and S levels were decreased below 50 IU/ml in 27 (10.2%) and 48 (18%) of patients. Both protein C and S levels were decreased in 14 (5.3%) of patients. 20 (74.1%) patients with protein C deficiency were on oral anticoagulation and only 7 (25.9%) were not on any anticoagulation. Similarly 35 (72.9%) of patients with protein S deficiency were on anticoagulation and only 13 (27.1%) of patients were not on anticoagulation and all 14 patients with decreased protein C and S levels were on anticoagulation. In effect only 7/266 (2.6%) or 13/266 (4.8%) patients had a decreased protein C or S level not attributed to anticoagulation.

Antithrombin

The median antithrombin level in this group was 109 % (12-778). Levels less than 80 %, considered as decreased were seen in 23 (8.6%) of patients. Three of these patients were on parenteral anticoagulation (UFH-2 and LMWH-1) at the time of evaluation. Thus in 20/266 (7.5%) patients, low AT levels could be considered a true risk factor.

Activated protein C ratio

The median APC ratio was 2.78 (1.06-5.48) and activated protein C resistance, revealed by a reduced APC ratio (< 2) was seen in 15 (6%) of patients (Table 7).

Table 7: APCR and factor V Leiden mutation

	Normal APCR		**Shortened APCR**		**Total**	
	No	**%**	**No**	**%**	**No**	**%**
Negative	174	93.0	13	7.0	187	92.1
Heterozygous	14	93.3	1	6.7	15	7.4
Homozygous	-	-	1	100.0	1	0.5
Total	**188**	**92.6**	**15**	**7.4**	**203**	**100**

Factor V Leiden mutation was the cause of this resistance in only 2/15 (13.4%) of patients. Similarly of the 16 patients positive for factor V Leiden mutation only 2 (12.5 %) had activated protein C resistance.

DNA mutations predisposing thrombosis

DNA markers for thrombosis was done in 203 (76.3%) of patients. The DNA markers evaluated were Factor V Leiden mutation, Prothrombin G20210A mutation and MTHFR Cys677Thr polymorphism. It has been well documented that only MTHFR 677TT (homozygous) genotype contributes a significant amount to hyperhomocysteinemia.[161,162] Hence the homozygous genotype alone was considered as a risk factor. There were no risk factors identified in 133 (65.5%) of patients and mutations were detected in 25 (12.3%) of patients. The incidence of Factor V Leiden mutation was 16/203 (7.9%),

prothrombin mutation was 2/203 (1.0%) and MTHFR mutation was 7/203 (3.4%). One patient was homozygous for factor V Leiden mutation and 7 patients for MTHFR homozygous 677TT mutation (Table 8).

Table 8: DNA mutations predisposing thrombosis

DNA marker	Status	Number (n-203)	%
V Leiden	**Heterozygous**	15	7.4
	Homozygous	1	0.5
Prothrombin	**Heterozygous**	2	1.0
MTHFR	**Homozygous**	7	3.4
Total		**25/ 203**	**12.3**

Sickle cell disease and PNH

There were no patients with PNH and only 2 (0.8%) patients with sickle cell disease and thrombosis in this group.

Table 9: Lupus anticoagulant (Dilute Russell's viper venom time-DRVVT)

DRVVT	Number	%	Cumulative %
Moderate positive	14	5.3	
Marked Positive	7	2.6	7.9
Mild positive *	68	25.6	25.6*
Negative	177	66.5	66.5
Total	266	100.0	100.0

* 25.6% patients with mild positive DRVVT were excluded because the test was to be repeated.

Acquired and inherited risk factors

DVT rarely occurs in the absence of risk factors and these risk factors are cumulative in their effect. The risk factors evaluated in our study were

Acquired risk factors
History of recurrence
Positive family history
Protein C deficiency
Protein S deficiency
Antithrombin deficiency
7. Factor VIII > 250 Iu/dL
8. Hyperfibrinogenemia
9. Factor V Leiden mutation
10. Prothrombin G20210A mutation
11. Homozygous MTHFR mutation
12. Activated protein C resistance

Table 10: Multiple risk factors

Number of risk factors	Frequency	%	Cumulative		Cumulative %
			Risks	No	
6	1	0.4	> 5	1	0.4
5	3	1.1	> 4	4	1.5
4	7	2.6	> 3	11	4.1
3	31	11.6	> 2	42	15.8
2	62	23.3	> 1	104	39.1
1	59	22.3			
Subtotal of patients with risk factors	**163**	**61.3**			
0- No risk factors	103	38.7			
Total	**266**	**100.0**			

In our study 163/266 (61.3%) of patients had at least one risk factor. 59/266 (22.3%) had only one risk factor, while 104/266 (39.1%) had more than 1 risk factors. 62/266 (23.3%) had 2 risk factors, 31/266 (11.6%) had 3 risk factors, 7/266 (2.6%) had 4 risk factors, 3/266 (1.1%) had 5 risk factors and 1/266 (0.4%) had 6 risk factors (Table 10).

Subgroup analysis of patients with venous thromboembolism

Between the year January 2005 and June 2007, a total of 154 patients had venous thromboembolism (Deep vein thrombosis + pulmonary embolism). These patients were evaluated separately for risk factors of thrombosis. The median age of presentation was 42 years (range: 8-74). There were 96 (62.3%) males and 58 (37.7%) females in this subgroup (Table 12). This study group contained 27 (17.5%) patients with history of recurrent thrombosis and 7 (4.5%) patients with family history of thrombosis (Table 11).

Table 11: History of recurrence and family history

	Recurrence history		**Family history**	
	Number	**%**	**Number**	**%**
Present	27	17.5	7	4.5
Absent	127	82.5	147	95.5
Total	**154**	**100**	**154**	**100**

In our group 70 (45.5%) patients were on anticoagulant therapy. Of these 48 (68.6%) patients were on oral and 22 (31.4%) were on parenteral anticoagulation (Table 12).

Table 12: Patients on anticoagulation

Anticoagulation		**Number**		**%**	
Oral		48		68.6	
Parenteral	**LMWH**	22	13	31.4	18.6
	UFH		9		12.9
Total		**70**		**100**	

Blood counts

The median hemoglobin of our whole group was 12 gm% (5.2 – 19.5), total white cell count was 7900/cmm (1300-57800), and platelet count 267000/cmm (12,000-12, 74,000).

Plasma coagulation tests

The median prothrombin time of patients in this study group was 12.6 (9.1-104.3) sec, activated partial thromboplastin time was 30.3 (22.8-166.5) sec and thrombin time was 14 (10.7-18) sec. Activated partial thromboplastin time was shortened below the normal range in 5 (3.2%) of patients.

Plasma coagulation factors

Plasma coagulation factors evaluated in this group of patients were factor VIII and fibrinogen. The mean factor VIII level in this study group was 275% (74-725) and fibrinogen level was 304 mg% (156-649). Hyperfibrinogenemia with levels of fibrinogen more than 450 mg% was seen in 20 (13%) patients. 90/154 (58.4%) of patients had factor VIII levels more than 250 Iu/dL. 27/154 (17.5%) patients had levels more than 450%, 24/154 (15.6%) had levels ranging between 351-450%, 27/154 (17.5%) patients had levels between 251-350%.

Natural inhibitors of coagulation

Protein C and S system

The median protein C level in this group was 98 IU/ml (10-171) and that of protein S was 78 Iu/ml (12-157). Protein C and S levels were decreased below 50 IU/ml in 12 (7.8%) and 28 (18.2%) of patients. Both protein C and S levels were decreased in 6 (3.9%) of patients. 9 patients with protein C deficiency were on oral anticoagulation and only 3 were not on any anticoagulation. Similarly 17 of patients with protein S deficiency were on anticoagulation and 11 patients were not on anticoagulation and all 6 patients with decreased protein C and S levels were on anticoagulation. In effect only 3/154 (1.9%) or

11/154 (7.1%) patients had a decreased protein C or S level not attributed to anticoagulation.

Antithrombin

The median antithrombin level in this group was 108% (40-149). Levels less than 80%, considered as decreased were seen in 16 (10.4%) patients. Three of these patients were on parenteral anticoagulation (UFH-2 and LMWH-1) at the time of evaluation. Thus in 13/154 (8.4%) patients, low AT levels could be considered a true risk factor.

Activated protein C ratio

The median APC ratio was 2.79 (1.48-5.48) and activated protein C resistance, revealed by a reduced APC ratio (< 2) was seen in 6 (3.9%) of patients.

DNA mutations predisposing thrombosis

DNA markers for thrombosis was done in 111 (72.1%) of patients. There were no risk factors identified in 96 (86.5%) of patients and mutations were detected in 15 (13.5%) of patients. The incidence of Factor V Leiden mutation was 10/111 (9.0%), prothrombin G20210A mutation was 1/111 (0.9%) and homozygous MTHFR mutation was 4/111 (3.6%). (Table 13)

Table 13: DNA mutations predisposing thrombosis

DNA marker	Status	Number (n-111)	%
V Leiden	**Heterozygous**	10	9.0
Prothrombin	**Heterozygous**	1	0.9
MTHFR	**Homozygous**	4	3.6
Total		**15/ 111**	**13.5**

Sickle cell disease and PNH

There were no patients with PNH and only 1 (0.6%) patient with sickle cell disease and thrombosis in this group.

Lupus anticoagulant (Dilute Russell's viper venom time-DRVVT)

Table 14: Lupus anticoagulant

DRVVT	Number	%	Cumulative %
Moderate positive	11	7.1	
Marked Positive	6	3.9	11.0
Mild positive*	39	25.4	25.4*
Negative	98	63.6	63.6
Total	**154**	**100.0**	**100.0**

* 25.4% patients with mild positive DRVVT were excluded because the test was to be repeated.

Acquired and inherited risk factors in patients with VTE

In 154 patients with venous thromboembolism, 97 (63%) had at least one risk factor. 34/154 (22.2%) had only one risk factor, while 63/154 (40.9%) had more than 1 risk factor. 39/154 (25.3%) had 2 risk factors, 21/154 (13.6%) had 3 risk factors, 2/154 (1.3%) had 4 risk factors and 1/154 (0.6%) had 5 risk factors. More than 2, 3 and 4 risk factors were seen in 15.6%, 1.9% and 0.6% of patients respectively (Table 15).

Table 15: Multiple risk factors

No of risk factors	Frequency	%	Cumulative		Cumulative %
			Risks	No	
6	0	0	> 5	0	0
5	1	0.6	> 4	1	0.6
4	2	1.3	> 3	3	1.9
3	21	13.6	> 2	24	15.6
2	39	25.3	> 1	63	40.9
1	34	22.2			
0	57	37.0			
Total	**154**	**100.0**			

Subgroup analysis of patients with cortical sinus thrombosis

Between the year January 2005 and June 2007, a total of 92/266 (34.6%) patients had thrombosis involving the cortical sinus system, documented with medical resonance imaging with or without angiogram, and were analyzed separately. The median age of presentation was 33 years (range: 4-65). There were 34 (37%) males and 58 (63%) females in this subgroup. A positive family history of thrombosis was there in 2 (2.2%) patients in this subgroup and history of recurrence was present in 9 (9.8%) of patients.

Counts and plasma coagulation tests

The median hemoglobin in this subgroup was 12 gm% (5.5 – 18.0), total white cell count was 8300/cmm (1600-23300), and platelet count 287000/cmm (10,000-777000).

The median prothrombin time of patients in this study group was 12.3 (8.9-83.8) sec, activated partial thromboplastin time was 30.1 (20.1-138.6) sec and thrombin time was 13.8 (11.0-24.2) sec.

Anticoagulation

In this subgroup 46 (50%) of patients were on some kind of anticoagulation. 29 (63%) patients were on oral anticoagulation and 17 (37%) were on parenteral (Low molecular weight-8 and un-fractionated heparin-7).

Plasma coagulation factors

The mean factor VIII level in this subgroup was 273 Iu/dL (83-719) and fibrinogen level was 307 Iu/dL (158-590). Hyperfibrinogenemia with levels of fibrinogen more than 450 mg% was seen in 8 (8.7%) patients. Elevated factor VIII levels (>250 Iu/dL) was seen in 56 (60.8%) of patients. Of patients with elevated factor VIII levels 17 (21%) patients had levels more than 450 Iu/dL, 13 (16%) had levels ranging between 351-450 Iu/dL and 26 (32.1%) patients had levels between 251-350 Iu/dL.

Natural anticoagulants

Protein C and S system

The median protein C level in this subgroup was 105 IU/ml (16-287) and that of protein S was 80 IU/ml (14-778). Protein C and S were decreased below 50 Iu/ml in 12 (13%) and 19 (20.7%) of patients. Both protein C and S were decreased in 8 (8.7%) of patients. 10/12 (83.3%) of patients with protein C deficiency were on oral anticoagulation. Similarly 17/18 (94.4%) of patients with protein S deficiency were on anticoagulation. All patients with decreased protein C and S levels were on anticoagulation. In effect only 2/92 (2.2%) patients with protein C deficiency and 1/92 (1.1) patient with protein S deficiency could not attributed to anticoagulation.

Antithrombin

The median antithrombin level in this group was 119% (40-188). Levels less than 80%, considered as decreased were seen in only 1 (1.1%) of patients. This patient was not on LMWH or UFH and this deficiency could not be attributed to this.

Activated protein C ratio

The median APC ratio was 2.26 (1.06-3.48) and activated protein C resistance, revealed by a reduced APC ratio (< 2) was seen in 4 (10.5%) of patients. Factor V Leiden mutation was the cause of this resistance in only 1/4 (25%) of patients.

DNA mutations predisposing thrombosis

DNA markers for thrombosis was done in 77 (83.7%) of patients. There were no risk factors identified in 69 (89.6%) of patients and mutations were detected in 8 (10.4%) of patients. All positive patients had only single mutations. The incidence of Factor V Leiden mutation was 4/77 (5.2%), prothrombin G20210A mutation was 1/77 (1.3%) and homozygous MTHFR Cys677Thr mutation was 3/77 (3.9%).

Lupus anticoagulant

Antiphospholipid antibody syndrome, due to lupus anticoagulant was positive in 3/92 (3.3%) of patients.

Acquired and inherited risk factors in patients with CST

In 92 patients with cortical sinus thrombosis, 53 (57.6%) had at least one risk factor. 20/92 (21.7%) had only one risk factor, while 33/92 (35.9%) had more than 1 risk factor. 28/92 (30.4%) had 2 risk factors, 3/92 (3.3%) had 3 risk factors and 2/92 (2.2%) had 4 risk factors. More than 2 and 3 risk factors were seen in 5.4% and 2.2% patients respectively (Table 16).

Table 16: Multiple risk factors

No of risk factors	Frequency	%	Cumulative		Cumulative %
			Risks	No	
6	0	0	> 5	0	0
5	0	0	> 4	0	0
4	2	2.2	> 3	2	2.2
3	3	3.3	> 2	5	5.4
2	28	30.4	> 1	33	35.9
1	20	21.7			
0	39	42.4			
Total	**92**	**100.0**			

Subgroup analysis of patients with Budd Chiari syndrome

Between the year January 2005 and June 2007, a total of 20/266 (7.5%) patients had Budd Chiari syndrome, due to thrombosis of the hepatic veins or the inferior vena cava and were analyzed separately. The median age of presentation was 30 years (range: 6-56). Males and females were equally represented in this subgroup. A positive family history of thrombosis was there in 1 (5%) patient in this subgroup and history of recurrence was present in 2 (10%) patients.

Counts and plasma coagulation tests

The median hemoglobin in this subgroup was 13 gm% (7.0 – 16.0), total white cell count was 7600/cmm (4000-17600), and platelet count 232000/cmm (43000-527000).

The median prothrombin time of patients in this study group was 14.5 (11.0-24.1) sec, activated partial thromboplastin time was 32.3 (23.4-49.5) sec and thrombin time was 14.4 (11.6-32.0) sec.

Anticoagulation

In this subgroup 5 (25%) of patients were on some kind of anticoagulation. 2 (40%) patients were on oral anticoagulation and 3 (60%) patients were on low molecular weight heparin.

Plasma coagulation factors

The mean factor VIII level in this subgroup was 299 Iu/dL (109-574) and fibrinogen level was 271 Iu/dL (170-375). Hyperfibrinogenemia was not seen in any patients in this subgroup. Elevated factor VIII levels (>250 Iu/dL) was seen in 13 (65%) of patients.

Natural anticoagulants

Protein C and S system

The median protein C level in this subgroup was 61 Iu/ml (17-212) and that of protein S was 94 Iu/ml (47-151). Protein C and S were decreased below 50 Iu/ml in 3/20 (15%) and 1/20 (5%) of patients. 1/3 (33.3%) of patients with protein C deficiency and the single patient with protein S deficiency were on oral anticoagulation. In effect only 2/20 (10%) patients with protein C deficiency could not attributed to anticoagulation.

Antithrombin

The median antithrombin level in this group was 87% (12-142). Levels less than 80%, considered as decreased were seen in 6 (30%) of patients. One of these 6 patients one was on unfractionated heparin and this deficiency could be attributed to this.

Activated protein C ratio

The median APC ratio was 2.95 (1.36-4.15) and activated protein C resistance, revealed by a reduced APC ratio (< 2) was seen in 1 (5%) of patients. Factor V Leiden mutation was the cause of this resistance in only 1/4 (25%) of patients.

DNA mutations predisposing thrombosis

DNA markers for thrombosis was done in 15 (75%) of patients. There were no risk factors identified in 13/15 (86.7%) of patients and mutations were detected in 2/15 (13.3%) of patients, both of whom were heterozygous for factor V Leiden mutation.

Lupus anticoagulant

Antiphospholipid antibody syndrome, due to lupus anticoagulant was positive in 1/20 (5%) of patients.

Acquired and inherited risk factors in patients with CST

In 20 patients with Budd Chiari syndrome, 13/20 (65%) had at least one risk factor. 2/20 (10%) had only one risk factor, while 11/20 (55%) had more than 1 risk factor.

The frequency of prothrombotic risk factors, both acquired and inherited are given in Table 17 and the clinical characteristics of patients in Table 18.

Table 17: Frequency of hereditary prothrombotic risk factors

	Whole group (n- 266)	**VTE (n-154)**	**CST (n-92)**	**BCS (n-20)**
Factor V Leiden	16/203* (7.9%)	10/111* (9.0%)	4/77* (5.2%)	2/15* (13.3%)
Prothrombin G20210A	2/203 (1.0%)	1/111 (0.9%)	1/77 (1.3%)	0
Homozygous MTHFR	7/203 (3.4%)	4/111 (3.6%)	3/77 (3.9%)	0
Protein C (<50 Iu/ml)	7/266 (2.6%)	3/154 (1.9%)	2/92 (2.2%)	2/20 (10.0%)
Protein S (<50 Iu/ml)	13/266 (4.8%)	11/154 (7.1%)	1/92 (1.1%)	0
Antithrombin III (<80 Iu/ml)	20/266 (7.5%)	13/154 (10.4%)	1/92 (1.1%)	5/20 (25.0%)
Elevated factor VIII (>250 %)	159/266 (59.7%)	90/154 (58.4%)	56/92 (60.8%)	13/20 (65.0%)
Elevated fibrinogen (>450mg %)	(10.5%) 28/266	20/154 (13.0%)	8/92 (8.7%)	0
Sickle cell anemia	2/266 (0.7%)	2/154 (1.2%)	0	0
Thalassemia	2/266 (0.7%)	2/154 (1.2%)	0	0

* Number of patients where DNA markers were done

Table 18: Overview of results

Parameters		**Whole group**	**VTE**	**CST**	**BCS**
Total No	*No.*	266	154	92	20
Median age	*Yrs*	38	42	33	30
Males	*%*	52.6	62.3	37	50
Family history	*%*	3.8	4.5	2.2	5.0
Recurrence	*%*	14.3	17.5	9.8	10.0
Anticoagulation	*%*	45.5	45.5	50	25
Oral	*%*	29.7	31.2	31.5	10
Heparin	*%*	15.8	14.3	18.5	15
Hb- Median (range: 5.2-19.5)	*gm/dl*	12	12	12	13
TC- Median (range: 1300-57800)	*No*	8200	7900	8300	7600
Platelets- Median (range: 0.1-12.7)	*x* 10^9	2.74	2.67	2.87	2.32
Coagulation tests					
PT	*Sec*	12.6	12.6	12.3	14.5
aPTT	*Sec*	30.4	30.3	30.1	32.3
TT	*Sec*	13.9	14.0	13.8	14.4

Subgroup analysis of patients with recurrent thrombosis

Between the year January 2005 and June 2007, a total of 38/266 (14.3%) patients with documented thrombosis had history of recurrent episodes of thrombosis in the past and were analyzed separately. The median age of presentation was 43 years (range: 21-73). There were 25 (65.8%) males and 13 (34.2%) females in this subgroup. A positive family history of thrombosis was there in 5 (13.2%) patients in this subgroup.

Thrombosis in the deep veins of the limbs were the most common site of thrombosis in this subgroup also, seen in 25 (65.8%) of patients, followed by cortical sinus thrombosis in 9 (23.7%) of patients. Pulmonary embolism as the presenting complains and Budd Chiari syndrome with thrombosis of the hepatic veins or inferior vena cava were seen in 2 (5.3%) patients each (Table 21).

Table 19: Site of thrombosis at presentation

Site	Number of patients	%
Deep vein thrombosis (DVT)	25	65.8
Cortical sinus thrombosis (CST)	9	23.7
Pulmonary embolism (PE)	2	5.3
Budd Chiari syndrome (BCS)	2	5.3
Total	38	100

Counts and plasma coagulation tests

The median hemoglobin in this subgroup was 13 gm% (5.2 – 16.0), total white cell count was 7500/cmm (1300-19600), and platelet count 303000/cmm (10,000-693000).

The median prothrombin time of patients in this study group was 13.3 (10.2-81.0) sec, activated partial thromboplastin time was 32.2 (23.4-56.3) sec and thrombin time was 13.6 (11.4-16.7) sec.

Anticoagulation

In this subgroup 17 (44.7%) of patients were on anticoagulant therapy. 14 (82.4%) patients were on oral anticoagulation and 3 (17.6%) were on parenteral (Low molecular weight or un-fractionated heparin) anticoagulation.

Plasma coagulation factors

The mean factor VIII level in this subgroup was 322 Iu/dL (133-694) and fibrinogen level was 307 Iu/dL (158-460). Hyperfibrinogenemia with levels of fibrinogen more than 450 mg% was seen in only 2 (5.3%) patients. Elevated factor VIII levels (>250 Iu/dL) was seen in 25/38 (65.8%) of patients. Of patients with elevated factor VIII levels 7 (18.4%) patients had levels more than 450 Iu/dL, 6 (15.8%) had levels ranging between 351-450 Iu/dL and 12 (31.6%) patients had levels between 251-350 Iu/dL.

Natural anticoagulants

Protein C and S system

The median protein C level in this subgroup was 100 Iu/ml (10-159) and that of protein S was 77 Iu/ml (12-156). Protein C and S were decreased below 50 Iu/ml in 4 (10.5%) and 11 (28.9%) of patients respectively. Both protein C and S were decreased in 3 (7.8%) patients. All 4 patients with protein C deficiency were on oral anticoagulation. Similarly 6 (54.5%) of patients with protein S deficiency were on anticoagulation and only 5 (45.5%) patients were not on anticoagulation and all patients with decreased protein C and S levels were on anticoagulation. In effect only 5/38 (13.1%) patients had a decreased S level not attributed to anticoagulation.

Antithrombin

The median antithrombin level in this group was 107% (62-136). Levels less than 80%, considered as decreased were seen in only 1/38 (2.6%) patient. This patient was also on LMWH and this deficiency could be attributed to this.

Activated protein C ratio

The median APC ratio was 2.26 (1.06-3.48) and activated protein C resistance, revealed by a reduced APC ratio (< 2) was seen in 4 (10.5%) of patients. Factor V Leiden mutation was the cause of this resistance in only 1/4 (25%) of patients.

DNA markers for thrombosis

DNA markers for thrombosis were done in 25 (65.8%) patients. There were no risk factors identified in 19 (76%) patients and mutations were detected in 6 (24%) patients. All positive patients had only single mutations. The incidence of Factor V Leiden mutation was 4/25 (16%), prothrombin and homozygous MTHFR mutation were seen in 1/25 (4.0%) patient each.

Subgroup analysis of patients with positive family history

Between the year January 2005 and June 2007, a total of 10/266 (3.7%) patients with documented thrombosis had a positive family history of thrombosis and were analyzed separately. The median age of presentation was 42 years (range: 16 -73). Males and females were equally represented in this subgroup. History of recurrence was present in 5 (50%) patients in this subgroup.

Thrombosis in the deep veins of the limbs were the most common site of thrombosis in this subgroup also, seen in 7 (70%) of patients, followed by cortical sinus thrombosis in 2 (20%) of patients and Budd Chiari syndrome with thrombosis of inferior vena cava in 1 (10%) patients. 60% of patients were on anticoagulation at the time of evaluation.

Counts and plasma coagulation tests

The median hemoglobin in this subgroup was 13 gm% (7.8 – 17.0), total white cell count was 8400/cmm (4800-13600), and platelet count 260000/cmm (193,000-399000).

The median prothrombin time of patients in this study group was 14.0 (9.1-57.8) sec, activated partial thromboplastin time was 30.2 (24-56.3) sec and thrombin time was 13.8 (11.4-15.0) sec.

Plasma coagulation factors

The mean factor VIII level in this subgroup was 362% (209-534) and fibrinogen level was 325 mg% (253-465). Hyperfibrinogenemia with levels of fibrinogen more than 450 mg% was seen in 2 (20%) patients. Elevated factor VIII levels (>250 %) was seen in 9/10 (90%) patients. 2 (20%) patients had levels more than 450%, 3 (30%) had levels ranging between 351-450% and 4 (40%) patients had levels between 251-350%.

Natural anticoagulants

Protein C and S system

The median protein C level in this subgroup was 105 Iu/ml (59-159) and that of protein S was 97 Iu/ml (17-153). There was no patients with protein C deficiency and Protein S were decreased below 50 Iu/ml in 2 (20%) of patients. Both patients with protein S deficiency were on oral anticoagulation and cannot be considered as true deficiency.

Antithrombin

The median antithrombin level in this group was 109% (88-126). There were no patients with antithrombin deficiency.

Activated protein C ratio

The median APC ratio was 2.72 (1.95-3.40) and activated protein C resistance, revealed by a reduced APC ratio (< 2) was seen in only 1 (10%) of patient. Factor V Leiden mutation was not done for this patient.

DNA mutations predisposing thrombosis

DNA markers for thrombosis was done in 6 (60%) of patients. Mutations were negative in 4/6 (66.7%) patients and detected in 2/6 (33.3%) patients - one (16.7%) patient each with factor V Leiden and homozygous MTHFR Cys677Thr mutation.

DISCUSSION

The present study was undertaken to evaluate the risk factors in a selected cohort of patients with documented thrombosis. In this study patients referred for thrombophilia screening, either had venous thromboembolism (Deep vein thrombosis with or without pulmonary embolism), cortical sinus thrombosis and Budd Chiari syndrome (thrombosis of hepatic veins with or without inferior vena cava). This is to our knowledge the second largest study done in India and the first from south India. The first study in India was from western India and they had evaluated 432 patients.[179]

Clinical characteristics.

Venous thromboembolism (VTE)

154 patients were documented to have deep vein thrombosis with or without pulmonary embolism. The median age at presentation in this group was 42 (8-74) years. Males (62.3%) outnumbered females and the male: female ratio was 1.6:1. In a case control study from Italy also the mean age was 47.4 (18–84) years and males comprised 48% (40.6-55.4) of patients.[180] Similarly in two other case control studies, the median age was 41 (9-77) years and 47.4 (18-84) years. The male: Female ratios in the same two studies were 1:1.3 and 1:1.1.[183,181] Thromboembolic events are infrequent in children and are usually a secondary complication. In the present study children below 15 years of age were only 1.3%. The incidence of thrombosis in children range from 0.07 to 0.14 per 10,000 children in general population, but 5.3 per 10000 hospital admissions in children.[182] The peak of the thrombotic risk is evidenced in the age group younger than 1 year and during adolescence. Similarly in a retrospective, population-based study in

Olmsted County residents, which evaluated 2218 patients, there were only 4 patients in the less than 15 years age group.[183]

In this study 4.5% of patients had family history of thrombosis and 17.5% had history of recurrence. Venous thrombosis is often a chronic condition, with recurrence rates estimated at 5% to 7% annually after a first episode,[6] the risk is highest among those whose initial episode was associated with cancer, and lowest among those whose initial episode was associated with a temporary risk factor such as surgery. Hron et al were not able to demonstrate an association between a positive family history of thrombosis with risk of recurrent thrombosis among 829 patients without deficiencies of protein C, protein S, or antithrombin.[184] In another case control study by Fredrick et al the prevalence of recurrent history of thrombosis was 19%.[185]

Cortical sinus thrombosis

92 patients were documented to have cortical sinus thrombosis by MRI/MRA. The median age at presentation in this group was 33 (4-65) years. There were 67% females in this group and the male: female ratio was 1:0.6. ISCVT, a prospective multinational observational study had evaluated 624 consecutive patients with CVT. The Median age in this group was 37 yrs (18-86) and females constituted 74.5% of patients.[186] In another study - a Meta analysis in which 17 studies and 48 - 2285 patients were included, the median age ranged from 24 - 43 years.[187] In another prospective study done in Netherlands and United Kingdom, which evaluated 59 patients with CVT, the median age was 33 yrs (18-80) and 50/59 (84.7%) were females.[188]

Budd Chiari syndrome

20 patients in this study had Budd-Chiari syndrome (BCS), characterized by occlusion of hepatic outflow either at the level of the hepatic veins or inferior vena cava. Cases were documented by colour Doppler or angiogram. The median age of patients in this group was 30 yrs (6-56) and male: female ratio was 1:1. In a large multicenter population-based case-control study, 43 patients with BCS were evaluated. The median age of this group was 40 yrs (19-60) and male: female ratio was 1:0.6.[189] In another study, evaluating 63 patients with BCS, the median age was 35 yrs (12-66) and males were 23.8%.[190]

Thrombophilia risk factors

Acquired risk factors

Acquired risk factors for thrombophilia in our study were present in 22.2% of patients. 22.2% of patients with VTE, 20.6% patients with CST and 30% patients with BCS had acquired risk factors. Of note 11/92 (12%) patients with cortical sinus thrombosis were post partum. In a study from Italy,[191] 130 unrelated patients with CST were evaluated. Transient risk factors like oral contraceptives, hormone replacement therapy, pregnancy/puerperium, surgery; head trauma and infection were seen in almost 60% of patients, compared to only 16.3% in our group. The cause for this difference is not evident, but could be due to inadequate documentation of risk factors. Pregnancy/Puerperium was a transient risk factor in 6.2% of patients, compared to 12% of patients in this study. In patients with BCS the common acquired risk factors were past abdominal surgeries and use of oral contraceptives. In a large multi-centre case control study, evaluating patients with BCS, the common acquired risk factors were myeloproliferative disorder (28%), Lupus anticoagulant in 5%, cirrhosis in 14%, previous

abdominal surgeries in 23% and oral contraceptives in 28%. In the other study also > 50% of patients had documented myeloproliferative disorders. In our study abdominal surgeries and oral contraceptive use was seen in 20% of patients and chronic liver disease in 10% of patients. There were no cases of myeloproliferative disorders in our series and is different from studies in literature.

Lupus anticoagulant (Dilute Russell's viper venom time-DRVVT)

Lupus anticoagulant was seen in 7.9% of our patients. Lupus anticoagulant was present in 8.3% of patients in the study from western India. In some studies incidence as high as 28% have been documented.[192] In the Leiden Thrombophilia Study, a population-based case-control study designed to determine risk factors for deep venous thrombosis (DVT). Lupus anticoagulant (LAC) was measured in 473 patients and 472 control subjects. Four control subjects (0.9%) and 14 patients (3.1%) had a positive LAC.[193]

Inherited risk factors

Plasma coagulation factors

An elevated factor VIII level was the most common risk factor in this study group, and was seen in 59.7% of our patients. In studies from the west elevated FVIII:C (> 1.5 iu/ml) emerged as the single commonest abnormality detected in 25.4% of a group of 260 patients with venous thromboembolism in one study.[194] In another study, the prevalence of elevated plasma levels of factor VIIIc in 65 patients with a proven single episode and in 60 matched patients with documented recurrent venous thromboembolism were systematically studied. To minimize the influence of the acute phase, blood was obtained at least 6 months after the thromboembolic event and results were adjusted for fibrinogen and C-reactive protein and factor VIIIc was re-determined several years after the first

measurement in a subset of patients to evaluate the variability over time. In the control, single and recurrent episode group, the prevalence of plasma levels of factor VIIIc above 175 IU/dl (90th percentile of controls) were 10% (95% CI: 4 to 21%), 19% (95% CI: 10 to 30%) and 33% (95% CI: 22 to 47%), respectively. For each 10 IU/dl increment of factor VIIIc, the risk for a single and recurrent episode of venous thrombosis increased by 10% (95% CI: 0.9 to 21%) and 24% (95% CI: 11 to 38%), respectively.[179] In our subgroup analysis, the incidence of high factor VIII in patient with history of recurrence was 25/38 (65.8%). Known causes of FVIII elevation, such as the acute thrombotic event itself; inflammation; malignancy; liver, renal, or vascular disease; surgery; or pregnancy, could explain the high incidence in our group. Some groups investigated C reactive protein along with factor VIII to ascertain if the elevation was reactive or not. However in our study this was not done. In our study fibrinogen levels were evaluated and since fibrinogen is also raised in the above situations, we would expect hyperfibrinogenaemia in our study. However the same was found in only 10.5% of patients in our group. Hence it is possible, that the elevated factor VIII level in our study is probably significant. The genetic basis for increased coagulation factor VIII levels is not well understood at this time and needs to be investigated.

Natural inhibitors of anticoagulation and DNA markers of thrombosis

The incidence of Protein C, S and AT deficiency in our study group, not attributed to anticoagulation was 2.6%, 4.8% and 7.5% respectively. In patients with history of recurrence there were no patients with Protein C or AT deficiency, but 13.1% of patients had protein S deficiency.

Venous thromboembolism

In patients with VTE, the prevalence of protein C, S and AT deficiency was 1.9%, 7.1% and 10.4%. When these were compared to data from the west and from one study from western India, the prevalence of protein C, S and AT III deficiencies were higher in our study and the other study from western India.[182] However DNA markers of thrombosis are more prevalent in the west compared to both our data and the other from India. In our study 2 patients with prothrombin G20210A mutation were seen and none were reported in the study from the other study from India. (Table 22)

Table 22: Etiology of venous thromboembolism – Hereditary prothrombotic risk factors

Characteristics	**Margaglione et al[183]**	**Tripodi et al[184]**	**Ghosh et al[182] Western India**	**Present study South India**
No. of patients	175	605	432	154
Antithrombin	0.6%	1.7%	2.6	13/154(8.4 %)
Protein C	1.2%	1.5%	9.5	3/154 (7.1 %)
Protein S	0.6%	2.1%	6.5	11/154(10.4 %)
Factor V Leiden	16.6%	14.4%	3.0	10/111* (9.0 %)
Prothrombin G20210A	14.2%	8.3%	0	1/111* (0.9 %)
Homozygous MTHFR	Not available	Not available	1.2	4/111* (3.6%)
Factor VIII (>250 Iu/dl)	Not available	Not available	Not available	90/154 (58.4%)
Fibrinogen (>450mg %)	Not available	Not available	Not available	20/154 (13.0%)

***** Number of patients for whom DNA mutations were analyzed.

Table 23: Etiology of cortical sinus thrombosis – Hereditary prothrombotic risk factors

Characteristics	Martinelli et al[195]	Present study
No. of patients	63	92
Antithrombin	2.5%	1.1%
Protein C	5.2%	2.2%
Protein S	3.1%	1.1%
Factor V Leiden	12.4%	5.2%
Prothrombin G20210A	21.5%	1.3%

Table 24: Etiology of Budd Chiari syndrome – Hereditary prothrombotic risk factors

Characteristics	Janssen et al[193]	P Deltenre et al[194]	Present study
No. of patients	43	63	20
Antithrombin	0	0	25
Protein C	9.3	19.0	10 %
Protein S	0	6.7	0
Factor V Leiden	25.6	31.7	13.3 %
Prothrombin G20210A	4.7	6.4	0

SUMMARY

1. 266 patients with documented deep vein thrombosis were evaluated for thrombophilia risk factors.
2. Median age of presentation is 38 years (4-74) and only 4/266 (1.5%) were below 15 years of age.
3. History of recurrence and family history was present in 38/266 (14.3%) and 10/266 (3.8%) of patients, respectively.
4. Male: Female ratio was 1:1.1 (140/126) for the whole group. Males were more commonly affected than females in the subgroups also, except for patients with cortical sinus thrombosis, where male: female ratio was 0.6:1.
5. The presenting features were lower limb deep vein thrombosis in 123/266 (46.2%) patients, followed by cortical sinus thrombosis in 92/266 (34.6%), pulmonary embolism in 26/266 (9.8%) and Budd Chiari syndrome was seen in 20/266 (7.5%) of patients.
6. 121/266 (45.5%) of patients were on anticoagulant therapy at the time of evaluation.
7. Prothrombotic risk factors were present in 163/266 (61.3%) of patients and no risk factors were identified in 103/266 (38.7%) of patients.
8. More than one risk factor was seen in 104/266 (39.1%) of patients.
9. Most common risk factor identified in these patients was an elevated factor VIII level- seen in 159/266 (59.7%) of patients.
10. Factor V Leiden mutation was seen in 16/203 (7.9%) patients, Prothrombin G20210A mutation in 2/203 (1.0%) and MTHFR Cys677Thr homozygous mutations in 7/203 (3.4%) of patients evaluated for these parameters. In patients with Budd Chiari syndrome the prevalence of Factor V Leiden mutation was 2/15 (13.3%).
11. Protein C, S and antithrombin deficiency was present in 7/266 (2.6%), 13/266 (4.8%) and 20/266 (7.5%) respectively.

12. Acquired risk factors were seen in 55/266 (20.7%) patients. Common acquired risk factors included lupus anticoagulant in 21/266 (7.9%), post partum state in 12/266 (4.5%) and malignancies in 10/266 (3.7%) patients.

CONCLUSIONS

Among patients with venous thromboembolism, proximal lower limb veins are most commonly involved. At least one prothrombotic risk factor is identified in 61.3% of patients. The commonest risk factor identified is elevated factor VIII level. Other hereditary risk factors for thrombosis, which includes factor V Leiden mutation, Prothrombin G20210A mutation and MTHFR Cys677Thr homozygous mutation were seen but in less frequency than in the western population. Acquired causes were present in 20.7% of patients.

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